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Lupus nephritis

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CASE PRESENTATION

A 22-year-old woman was referred to the University Hospital St. Radboud eight years ago because of general fatigue, arthralgias, myalgias, facial rash, and a fever (38.1°C). Her general physician had prescribed salicylates and later nonsteroidal anti-inflammatory drugs, which partially relieved the symptoms. Several years before, systemic lupus erythematosus (SLE) had been diagnosed in one of her sisters who had had similar complaints.

Physical examination of this healthy-looking, white female revealed a typical butterfly rash on the face but no further skin abnormalities. Her blood pressure was 140/70 mm Hg supine and standing, and the pulse rate was 76 beats/min and regular. She had no signs of anemia or edema. Oral and genital ulcers were absent as was lymphadenopathy. None of the joints showed signs of synovial swelling. Auscultation did not reveal a pleural or a pericardial rub but did disclose a systolic murmur, grade II/VI, at the left fourth intercostal space and the apex. These findings suggested mitral valve insufficiency, which was confirmed on echocardiography. Further physical examination was unremarkable.

Laboratory examination revealed a sedimentation rate of 106 mm/hr; urea, 5.6 mmol/liter; creatinine, 91 μmol/liter (1.0 mg/dl); and serum albumin, 29 g/liter. A 24-hour urine collection contained 1.29 g of protein. The urinary sediment contained 5–10 polymorphic erythrocytes/high-powered field and numerous hyaline and red blood cell casts. The hemoglobin was 6.1 mmol/liter; leukocytes, 3.0×10^9/liter; and thrombocytes, 222×10^9/liter. The LDH value was elevated to 372 U/liter. Liver function tests all were normal. An antiglobulin test was strongly positive, both with an anti-IgG and an anti-complement antiserum. Serologic examination showed a strongly positive homogenous ANA, with a positive *Crithidia luciliae* test indicating the presence of anti-dsDNA antibodies. The Farr assay was strongly positive, with 5000 U/ml. The C3 and C4 levels were decreased to 559 and 99 mg/liter, respectively (normal values for C3: 750–1250 mg/liter; C4: 180–400 mg/liter). The immunoblot on nuclear extracts was diffusely positive but did not disclose specific bands. Tests for ANCA, rheumatoid factor, anti-cardiolipin, and cryoglobulins all were negative. A chest radiograph and renal ultrasound examination did not disclose any abnormalities. A percutaneous renal biopsy had to be postponed because of a prolonged bleeding time due to the nonsteroidal drugs. To relieve her arthralgias, prednisone (25 mg/day) was prescribed. Two weeks later a renal biopsy was performed under ultrasound guidance. The specimen contained 34 glomeruli, of which 40% showed capillary loop necrosis with fibrin deposits. Cellular crescents were present in some glomeruli. Endocapillary proliferation with influx of granulocytes and mononuclear cells was seen in 80% of the glomeruli. The interstitium contained a scarce, focal mononuclear cell infiltrate. Immunofluorescent examination revealed coarse granular deposits of IgG (2+), IgM (3+), IgA (1+), C1q (4+), and C3 (4+) along the glomerular capillary walls and to a lesser extent in the mesangium. Based on these findings, the biopsy was classified as lupus nephritis WHO class IV.B with an activity index of 18 and a chronicity index of 1.

After discussing the therapeutic options, the patient selected treatment with azathioprine and prednisone over cyclophosphamide and prednisone because of the lower risk of infertility with the former regimen. Therefore, the prednisone dose was raised to 60 mg/day, azathioprine was started at a daily dose of 125 mg (2 mg/kg), and ranitidine was added prophylactically. During this treatment, her complaints disappeared completely, the sedimentation rate fell from 106 to 30 mm/hr, and the serum creatinine decreased from 96 to 65 μmol/liter (1.1–0.7 mg/dl). The urinary protein excretion initially rose to 7.1 g/24 hr maximally, but thereafter decreased gradually to 0.8 g/24 hr, although it never became negative. Concomitantly, the serum albumin rose from 29 to 41 g/liter. Hematologic parameters improved and became normal. The serologic immunologic parameters also reflected the salutary effect of the treatment: the Farr titer dropped to 140 U/ml (Fig. 1), and the C3 (but not the C4) level became normal. The azathioprine dose was kept constant; the prednisone dose was tapered every 4 weeks by 10 mg/day until a dose of 30 mg/day was reached. Thereafter, the dose was tapered every 4 weeks by 5 mg/day to a maintenance dose of 10 mg/day, which was reached eight months after her initial presentation. The course of the disease was unremarkable for five months, until the urinary protein excretion again rose, from 2.0 to 7.4 g/24 hr, with a parallel decrease of her serum albumin from 44 to 33 g/liter. The Farr assay, which had been negative, became positive again at 12 months (see Fig. 1), and C3 decreased from 851 to 581 mg/liter. Clinically, she had no signs of disease activity.

We discussed with the patient the option of starting intravenous cyclophosphamide pulse therapy, but her fear of infertility prevented her approval. The oral prednisone dose therefore was increased to 30 mg/day, and she received three intravenous pulses of 1 g of methylprednisolone on three consecutive days; the azathioprine dose remained unchanged. With the new regimen, the proteinuria again fell to 2 g/24 hr, serum albumin rose to 38 g/liter, and the Farr assay became negative again. The prednisone dose was tapered as before. Within 12 months, the proteinuria had again increased, reaching nephrotic-range values, with a gradual decrease of the serum albumin level to 28 g/liter. Renal function deteriorated; the serum creatinine rose from 66 to 90 μmol/liter (0.7–1.0 mg/dl). The hemolytic anemia worsened, resulting in a hemoglobulin value of 5.8 mmol/liter. However, the white blood cell and platelet counts remained normal. As depicted in Fig. 1, the Farr assay again became positive at 23 months. Treatment with cyclophosphamide still was not acceptable to the

Key words: lupus nephritis, proteinuria, systemic lupus erythematosus.

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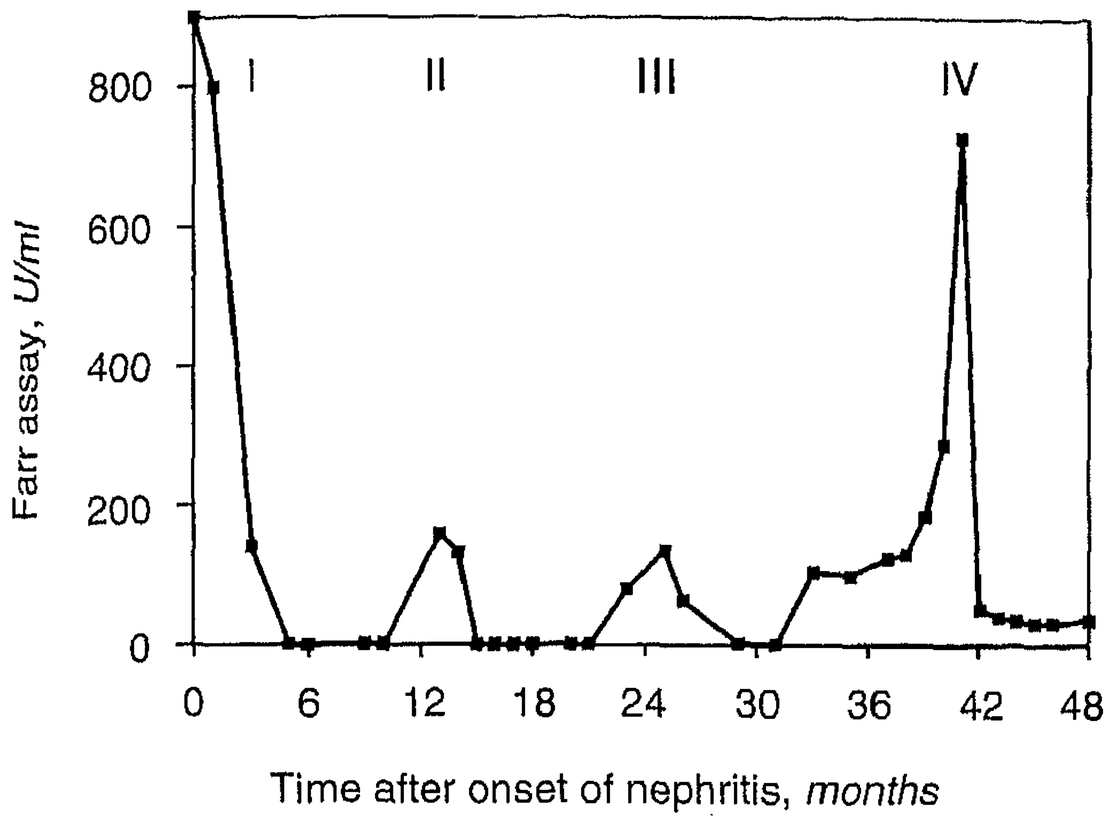


Fig. 1. Profile of the anti-dsDNA titers over time of the presented patient as measured in the Farr assay (expressed as U/ml). Roman numerals indicate onset (I) or renal exacerbations (II, III, IV).

patient, so the oral prednisone dose was raised from 10 mg/day to 60 mg/day. Furosemide was prescribed for her edema. After this round of intensification of treatment, the anti-dsDNA levels became negative, and the C3 and C4 values returned to normal. The proteinuria responded only partially (from 8 to 4 g/24 hr), although the serum albumin levels rose to 34 g/liter. By 34 months after presentation (prednisone dose, 10 mg; azathioprine dose, 125 mg), the serologic quiescence had ended; Farr titers became positive again (100 U/ml). This development, together with an increase in proteinuria and a decrease in serum albumin, led to the decision to discontinue the azathioprine and to start monthly intravenous pulses of cyclophosphamide. At months 37 and 38, she received 1 g of cyclophosphamide together with hydration, ondansetron, and MESNA (2-mercapto-ethane-sulfonate). Except for nausea after 48 hours, she tolerated this treatment well. The next dose of cyclophosphamide had to be postponed four weeks because of leukopenia. During that time, her renal condition deteriorated; the serum creatinine rose to 161 μmol/liter (1.8 mg/dl), the urinary protein excretion increased, and the serum albumin decreased further to 22 g/liter. Her blood pressure rose to 170/110 mm Hg, for which she was given atenolol. Two weeks after the third cyclophosphamide infusion, she was admitted to the hospital because her renal function had deteriorated further (serum creatinine, 259 μmol/liter). Also, an increase in dsDNA titers indicated that serologically the disease activity had increased (Fig. 1).

In an ultimate attempt to control the disease, the oral prednisone dose was increased again to 60 mg/day, azathioprine therapy was reinstituted, and plasmapheresis was started every other day for one week and thereafter thrice weekly for two weeks. Blood pressure control was improved by adding nifedipine and increasing the dose of furosemide. This regimen led to a stabilization of renal function, with a serum creatinine of about 300 μmol/liter (3.4 mg/dl); a rapid decline of anti-dsDNA antibody titers; and a normalization of serum albumin despite proteinuria of 8 g/24 hr. The prednisone dose was tapered as described. Beginning at 46 months, her renal function gradually deteriorated further, resulting in a creatinine level of 620 μmol/liter (7.2 mg/dl) (51 months post presentation) despite adequate blood pressure control, immunosuppressive maintenance therapy, and serologic disease quiescence. Standard measures were taken (protein restriction, oral phosphate binders, 1α-hydroxy-vitamin D_3, erythropoietin substitution) to control the manifestations of the progressive renal insufficiency. During extensive discussion, it was decided not to increase the immunosuppression and to taper the azathioprine and prednisone, thus accepting eventual progression to end-stage renal disease and opting for a renal transplant from a living-related donor.

Three months later, hemodialysis became necessary to control her azotemia. In the meantime, preparations for the transplantation procedure had been started. After blood group and HLA typing, her mother offered a kidney. After a third-party blood transfusion and repeatedly negative cross-matching thereafter, the renal transplant was performed three months after hemodialysis had been started. The graft functioned immediately, and the postoperative course was uneventful. Immunosuppression consisted of cyclosporine A and prednisone. The patient was discharged on the 8th postoperative day in excellent condition; her serum creatinine was 130 μmol/liter (1.5 mg/dl). Three months later, a percutaneous transplant biopsy was performed because of a rise of the serum creatinine (to 168 μmol/liter; 1.8 mg/dl), which could not be attributed to post-renal obstruction. This biopsy showed focally circumscribed areas of mononuclear cell infiltrates and edema in the interstitium. Mononuclear cells occasionally were present in the tubules. No glomerular abnormalities were apparent. According to the Banff classification [1] these findings were classified as "borderline interstitial rejection." There were no signs of cyclosporine A nephrotoxicity. The patient was treated with intravenous infusions of 1 g methylprednisolone on three consecutive days. The serum creatinine gradually decreased to 143 μmol/liter (1.6 mg/dl). Over the past four years, the serum creatinine has gradually risen again to its current value of 165 μmol/liter (1.8 mg/dl), but this increase paralleled her increase in body weight from 46 kg at transplantation to 70 kg at present. She currently is in excellent condition and has a full-time job. The urine contains no protein. Her blood pressure is 130/84 mm Hg; she takes 50 mg of atenolol daily. Her SLE has remained silent both clinically and serologically since end-stage renal failure developed four years ago.

DISCUSSION

DR. JO H. M. BERDEN (*Professor of Nephrology, Division of Nephrology, University Hospital St. Radboud, Nijmegen, The Netherlands*): This patient illustrates the severe renal manifestations complicating systemic lupus erythematosus. Like SLE itself, renal manifestations of the disease are highly variable in their clinical presentation, ranging from mild proteinuria to rapidly progressive glomerulonephritis causing renal insufficiency within weeks [2]. This clinical variability is in keeping with the broad spectrum of histologic abnormalities present in renal biopsy specimens from these patients. The histologic findings in lupus nephritis are categorized in six groups according to a recently modified World Health Organization (WHO) classification (Table 1) [3]. A recent Nephrology Forum specifically addressed the relation between pathologic abnormalities and clinical symptoms [4]. Using the case presented, I plan to focus on three other major aspects of SLE: (*1*) new insights into the pathophysiology of lupus nephritis, (*2*) treatment options in lupus nephritis, and (*3*) renal replacement therapy in SLE.

A central feature of all renal biopsies from patients with lupus nephritis is the presence on immunofluorescence examination of immunoglobulin and complement deposits present either in the mesangium (WHO class II) or in the mesangium and the capillary loops (WHO class III, IV, or V) [3]. For many years, these deposits were thought to be secondary to the deposition in the glomerulus of DNA/anti-DNA immune complexes, because in SLE anti-dsDNA antibodies are found in the circulation [5], onset or flares of renal manifestations often are preceded by a rise in titer of these anti-dsDNA antibodies [6] (Fig. 1), and anti-dsDNA antibodies can be eluted from glomeruli in lupus nephritis [7]. Twenty-five years ago, this concept even led Koffler et al to propose SLE, and more particularly lupus nephritis, as the prototype of an immune-complex disease [8]. Several crucial pieces of evidence are lacking to support this central role for DNA/anti-DNA complexes, however. Free DNA is not present in the circulation [9, 10], the presence of DNA/anti-DNA complexes in SLE is highly questionable [11], and glomerular localization is rarely observed in experimental animals after intravenous injection of artificially prepared DNA/anti-DNA complexes [9]. Therefore, alternative hypotheses have been developed.

The generation of monoclonal anti-DNA antibodies led to the

Table 1. World Health Organization morphologic classification of lupus nephritis (1995 revised version)[a]

Class	Description
I	Normal glomeruli
	A Nil (by all techniques)
	B Normal by light microscopy, but deposits seen by electron or immunofluorescence microscopy
II	Pure mesangial alterations (mesangiopathy)
	A Mesangial widening and/or mild hypercellularity (+)
	B Moderate hypercellularity (++)
III	Focal segmental glomerulonephritis (associated with mild or moderate mesangial alterations)
	A Active necrotizing lesions
	B Active and sclerosing lesions
	C Sclerosing lesions
IV	Diffuse glomerulonephritis (severe mesangial, endocapillary, or mesangiocapillary proliferation and/or extensive subendothelial deposits. Mesangial deposits are present invariably and subepithelial deposits often, and can be numerous).
	A Without segmental lesions
	B With active necrotizing lesions
	C With active and sclerosing lesions
	D With sclerosing lesions
V[b]	Diffuse membranous glomerulonephritis
	A Pure membranous glomerulonephritis
	B Associated with lesions of Category II (a or b)
VI	Advanced sclerosing glomerulonephritis

[a] From Ref. 3.

[b] In the previous version of this classification, membranous glomerulonephritis accompanied by proliferative lesions in the capillary loops was classified as V.C/V.D. In the modified version, these forms are categorized in class IV.

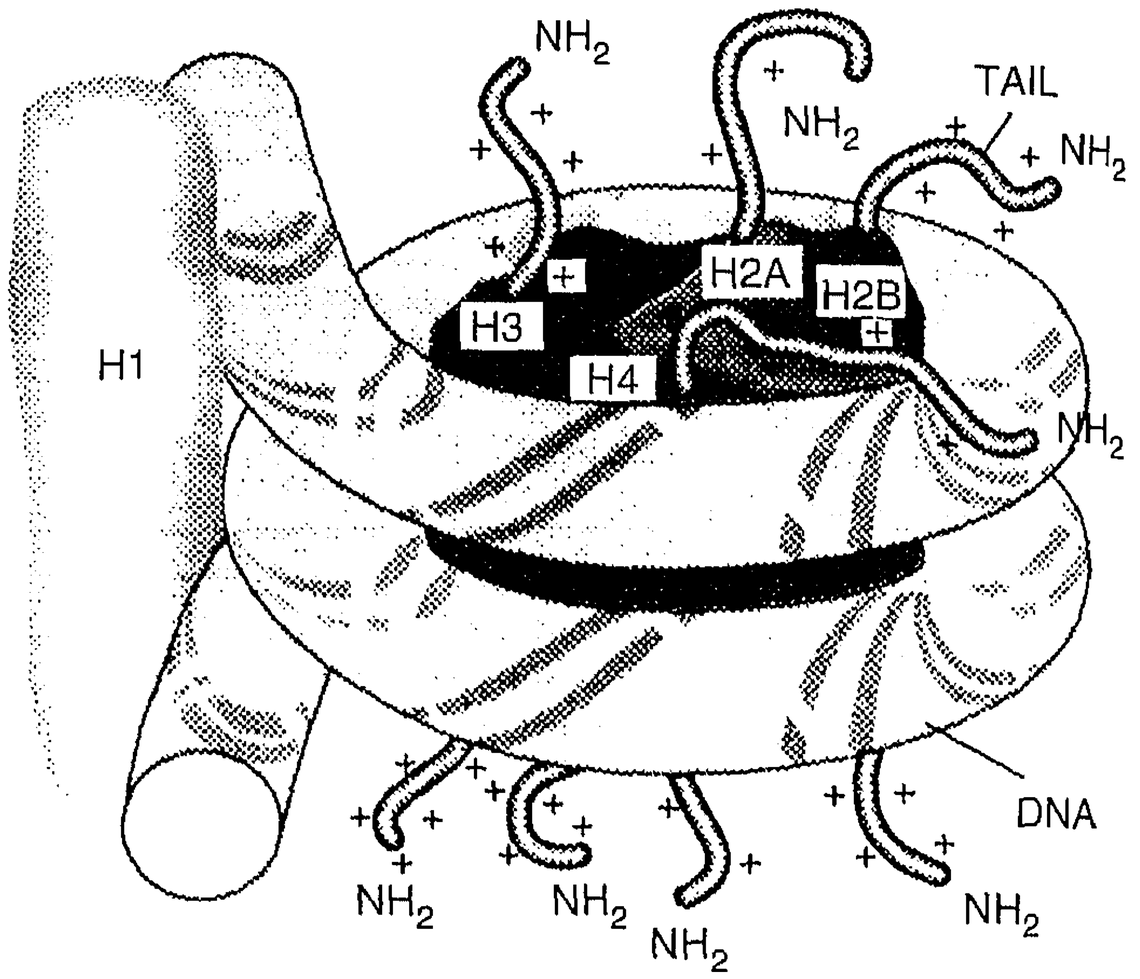


Fig. 2. Composition of nucleosome. The central part of the nucleosome is formed by the histone octamer, consisting of two pairs of the H2A-H2B dimer and one H3-H4 tetramer. Around this octamer 145 bp of DNA are wound in two superhelical turns. Histone H1 interacts with the octamer/DNA complex and bridges, together with stretches of linker DNA, adjacent nucleosomes. Note that the positively charged N-terminal regions of the core histones are located at the outside of the nucleosome. (Reproduced with permission from *Scientific American*. International Edition, October 1992, from the article "Histones as regulators of genes" by M. Grunstein, 40–47.)

discovery that some of these monoclonals could react with non-DNA antigens [12], including intrinsic glomerular basement membrane (GBM) constituents like heparan sulfate [13] and laminin [14]. These observations suggested that anti-DNA antibodies could directly bind to the GBM [9, 15]. Subsequently, however, it was demonstrated that histone/DNA (nucleosome) complexes mediate the binding of these antibodies to cell surfaces [16], to heparan sulfate [17], to laminin [18], and to endothelial and mesangial cells [19]. The hypothesis then arose that the binding of anti-DNA antibodies to the GBM in vivo was not direct, but indirect, mediated by nucleosomes [20]. According to this concept, the cationic histone part of the nucleosome is responsible for the binding to heparan sulfate and to other anionic determinants in the GBM. This concept was proven experimentally by renal perfusion in vivo in rats of monoclonal anti-nuclear (anti-DNA and anti-nucleosome) antibodies complexed to nucleosomes, which indeed led to GBM binding, in contrast to lack of GBM binding when non-complexed antibodies were utilized [21]. However, nucleosomes not only appeared to be important for the evolution of tissue lesions and glomerulonephritis in particular, but also for the induction of the autoimmune response in SLE. Strong evidence indicates that the autoimmune response in SLE is (auto)antigen driven and T-cell dependent [22–24]. This conclusion is based on the following observations regarding anti-dsDNA antibodies: (*1*) they are somatically mutated, (*2*) they show a restricted rather than a random expression of VH and VL gene products, (*3*) different individuals and different species share common idiotypes, (*4*) they show a class switch to IgG and predominantly to the T-cell-dependent IgG2a/2b isotypes, and (*5*) it appears that $CD4^+$ T-helper cells are important for their formation [24]. However, it is difficult to understand how DNA can induce an autoimmune response, because the T-cell receptor does not recognize oligonucleotides or naked DNA, but only processed peptides presented in the groove of the MHC class-II molecule on antigen-presenting cells. Indeed, numerous reports have shown that naked DNA itself is very poorly immunogenic [reviewed in Ref. 24]. A major breakthrough was achieved when it was found that the tolerance for dsDNA could be broken by immunization of DNA complexed to DNA-binding proteins like histones [reviewed in Ref. 25]. Thus the association with histones appears to be critical for the formation of anti-dsDNA antibodies. These observations provided the first indication that nucleosomes are important as triggering autoantigens in SLE [23, 26, 27].

New insights into the pathogenesis of lupus nephritis

Nucleosomes. Before addressing the relevance of nucleosomes both for the induction and the effector phase in SLE, I want to briefly review the structure of nucleosomes. The nucleosome, the basic structure of chromatin, has an important function in the compaction of DNA in the nucleus. It consists of pairs of the 4 core histones—H2A, H2B, H3, and H4—forming the histone-octamer around which 145 bp of DNA are wound twice (Fig. 2). Histone H1 anchors these DNA loops. Neighboring nucleosomes are connected with histone-free linker DNA. This architecture is responsible for the characteristic appearance of chromatin in electron microscopy as "beads-on-a-string." Each of the 4 core histones is a single polypeptide chain with a molecular weight between 11 and 15 kD. Basic residues, which are clustered at the

N-terminal regions of the core histones and therefore located at the outside of the nucleosome, create regions with a strong positive charge (Fig. 2). The positive charges on the cylindrical surface of the histone octamer are partly involved in DNA binding. But these regions of positive charges within the nucleosome also are important for binding to anionic moieties like heparan sulfate in the GBM, as we will discuss later.

As I said, naked DNA is not present in the circulation [9], but in 1990 Rumore and Steinman showed that DNA does circulate in the form of (oligo-) nucleosomes [28]. The presence of DNA in this particular form suggests that apoptosis is the source for the generation of these nucleosomes. Apoptosis, a process of programmed cell death, leads to a non-phlogistic removal of "unwanted" cells. In contrast to accidental cell death by necrosis, apoptosis can yield nucleosomes, because it starts with an internucleosomal cleavage of chromatin [29]. This nucleosomal material together with spliceosomes (snRNP/Sm) is clustered in the so-called "apoptotic bodies," which appear at the surface of the cell during apoptosis. Normally, apoptotic cells are phagocytosed rapidly both by macrophages and by neighboring parenchymal cells. This phagocytosis efficiently prevents the release of phlogistic cell constituents into the micro-environment [30]. If apoptosis or phagocytosis is disturbed, however, or if apoptosis occurs in the circulation, nucleosomes can be released systemically. Under in-vitro conditions in which effective phagocytosis is absent, the amount of nucleosomes released strongly correlates with the degree of apoptosis [31]. Apoptosis of lymphocytes can be induced by several mechanisms, including T-cell receptor/CD3 activation or binding of the Fas receptor to its ligand [32]. This Fas receptor, belonging to the TNF receptor superfamily, is not expressed in the spontaneous lupus model, the MRL/lpr mouse [33]. This observation for the first time linked disturbed apoptosis to SLE. Studies later reported that the defective expression of the Fas ligand in the gld mouse had the same consequences, namely, lymphoproliferation and systemic autoimmunity [34, 35]. Correction of the defective expression in both models by transgenic techniques prevented the development of autoimmunity [36]. Overexpression of bcl-2, a physiologic inhibitor of apoptosis, also leads to autoimmune features. It reduces the elimination of autoreactive B-cells [37], induces the formation of anti-nucleosome antibodies, and leads to the development of immune-complex glomerulonephritis [38]. In human SLE, the expression of the Fas system is normal, but the increased concentrations of soluble Fas (which inhibits apoptosis) reported by some [39] but not all [40, 41] investigators, the increased expression of bcl-2 in T-cells in lupus patients [42], and the increased rate of apoptosis in vitro [31] point to abnormalities of apoptosis also.

These disturbances in apoptosis can have two major consequences. First, they can lead to the persistence of autoreactive T-cells [43], since apoptosis is the major mechanism for shaping the T-cell repertoire in the thymus [32]. Second, they might lead to quantitative and qualitative changes in the release of nucleosomes. This increased release of nucleosomes might be further augmented by defects in phagocytosis of apoptotic cells or by impairment of C-reactive protein-mediated clearance of nucleosomes [44–46]. Both processes are disturbed in SLE. Furthermore, abnormalities in apoptosis can produce structural changes in released nucleosomes, via oxygen-free-radical-induced protein modification or fragmentation [47, 48]. This effect might create novel epitopes within the nucleosome that induce activation of non-tolerant T-helper cells. The causative relationship between apoptosis and autoantibody formation was demonstrated by the observation that lupus-derived, nucleosome-specific autoantibodies bind to nucleosomes either present in the apoptotic bodies [47] or released from apoptotic cells [49]. In summary, it seems that in genetically susceptible individuals, disturbances in apoptosis can contribute to the development of SLE.

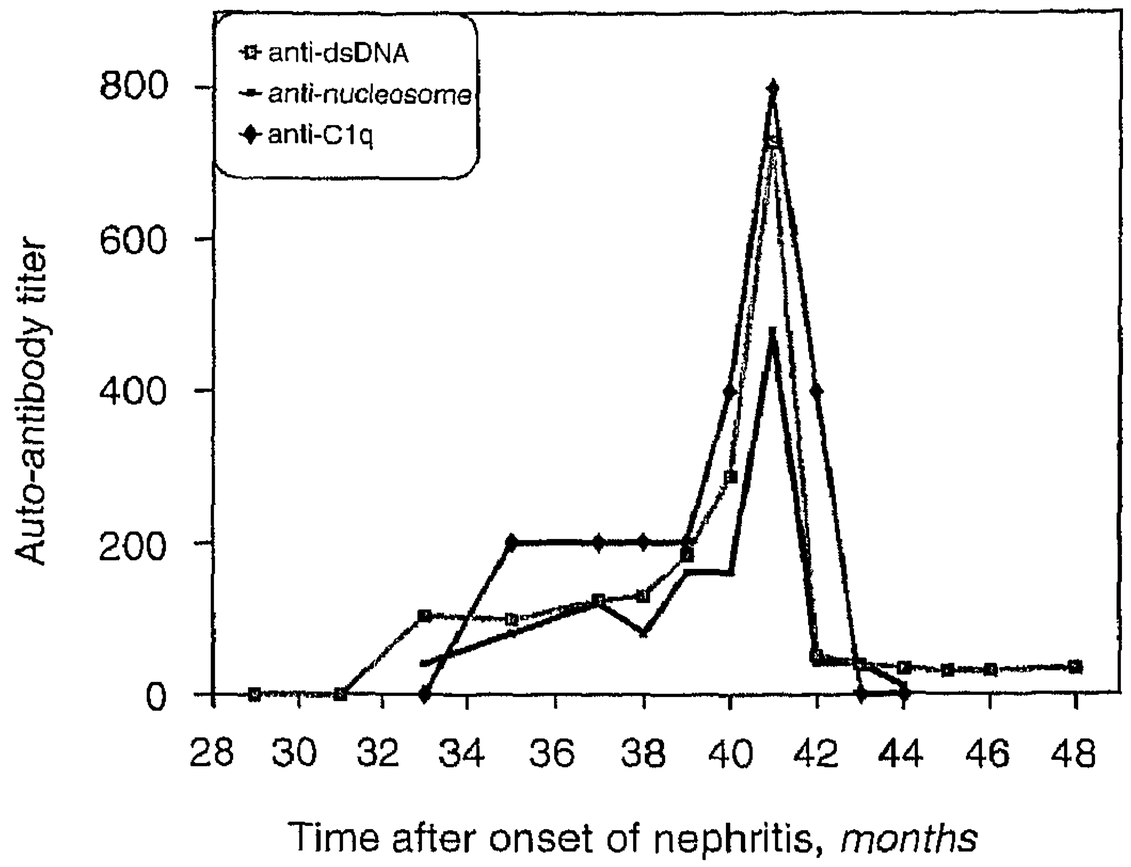


Fig. 3. Profile of anti-dsDNA, anti-C1q, and nucleosome-specific antibodies measured during the last renal exacerbation (episode IV in Fig. 1) of the patient. Results are expressed as U/ml for the Farr assay and as titers for the two other autoantibody specificities. For the latter, the titers are the reciprocals of the dilutions that give an absorption of 1.0 at 450 nm in the ELISA.

Nucleosomes are probably the major autoantigens that drive the autoimmune response in SLE. *First*, Bell and coworkers showed that nucleosomes can act as polyclonal B-cell activators [50]. More important, since SLE is an antigen-driven disease, nucleosomes, in contrast to dsDNA or histones, can stimulate $CD4^+$ T-helper cells in murine lupus [51]. The same authors also observed that these nucleosome-specific T-helper cells could induce the production of anti-dsDNA and anti-histone antibodies by syngeneic B-cells. Mamula recently suggested that this epitope spreading is explained by the presentation to T-cells of previously cryptic epitopes unmasked during processing by antigen-presenting cells [52]. *Second*, the formation of nucleosome-specific antibodies, that is, antibodies that react exclusively with the nucleosome, but not with its constituents DNA or histones, is reported with increasing frequency in SLE [reviewed in Ref. 53] and occurs in as many as 80% of lupus patients [26, 54]. In our patient, we retrospectively measured the anti-nucleosome titer in plasma samples set aside during her last relapse (episode IV in Fig. 1). As Figure 3 illustrates, an increase in nucleosome-specific antibodies that mimicked the course of the anti-dsDNA titer accompanied this last renal flare. Unfortunately no blood samples were available from the initial phase of her disease. Studies show that the formation of these nucleosome-specific antibodies precedes the formation of antibodies with other anti-nuclear specificity, for example, anti-dsDNA and anti-histone [26, 55, 56]. At present not enough data exist to relate nucleosome-specific antibodies to certain SLE disease manifestations such as lupus nephritis. Until now either a positive association [26, 49] or none at all [57] has

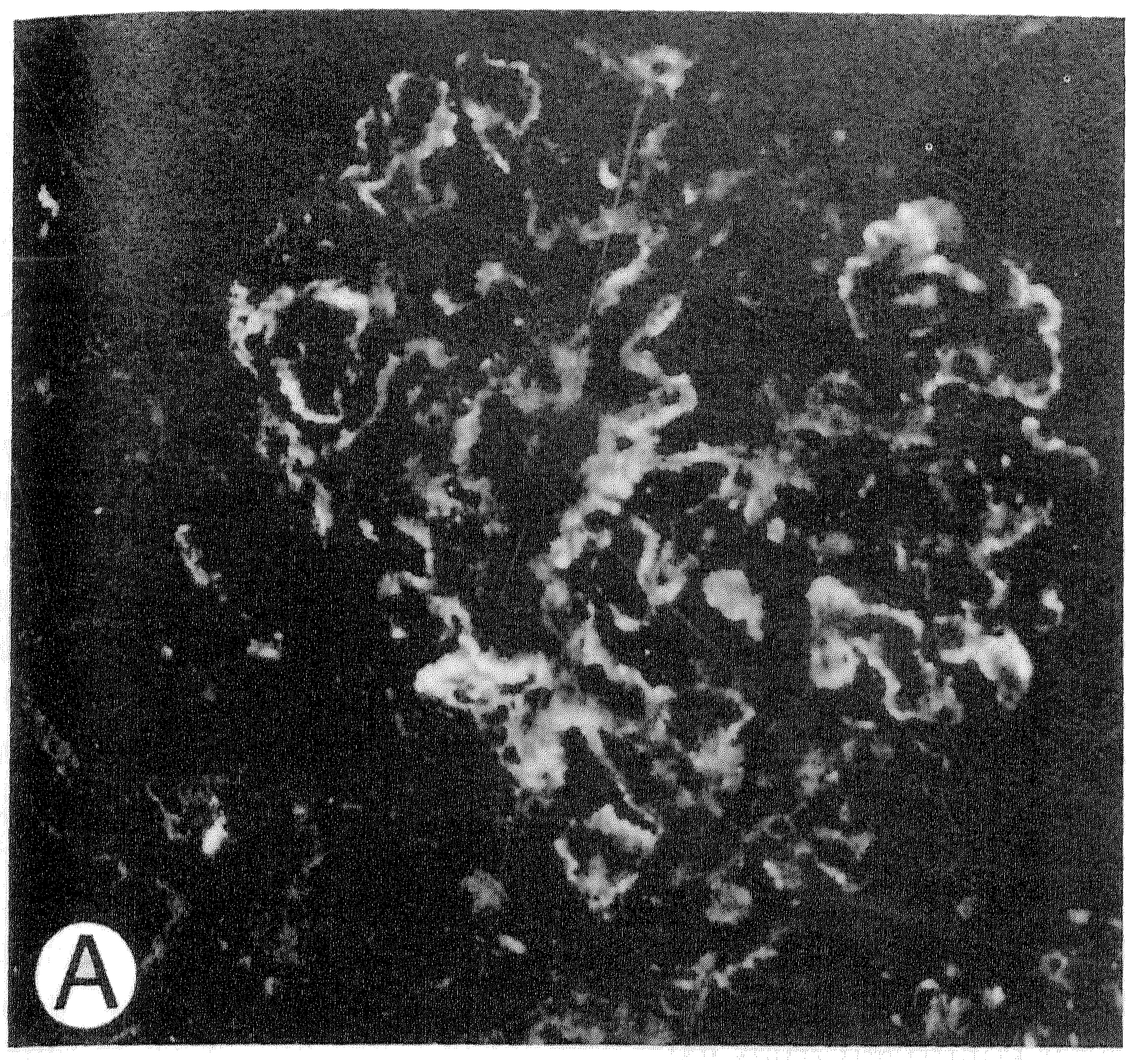
been described. *Third*, a number of indications suggest that the incidence of nucleosome-specific antibodies in SLE is higher than thought because some anti-DNA antibodies are, in fact, nucleosome-specific antibodies. Anti-nucleosome antibodies complexed to nucleosomal antigens can display anti-DNA reactivity in the dsDNA-ELISA, but more important, also in the Farr assay [21], which is regarded as the "gold standard" for the detection of anti-DNA antibodies [58]. This DNA reactivity is caused by the cationic histone within the nucleosome, complexed to the autoantibody. Removal of the bound nucleosomes abrogated this DNA reactivity, but the nucleosome reactivity remained. This phenomenon was first observed for monoclonal antibodies, but evidence now suggests that it also applies to polyclonal antibodies from patients with lupus. Exposure of Farr-positive blood samples from SLE patients to high-salt conditions and DNAse reduced Farr reactivity by 70%; reactivity towards other autoantigens (nucleosomes/SS-A) or control antigens (EBV) remained unaltered [59]. Another indication that anti-DNA reactivity might be due to nucleosome-specific antibodies comes from the observation that monoclonals with a high affinity for dsDNA bind even better to nucleosomes than to dsDNA, as revealed by the measurement of equilibrium affinity constants in a sensitive system [60]. Taken together, these data indicate the importance of the nucleosome as a dominant driving autoantigen in lupus, and suggest that the loss of tolerance for nucleosomes is an initial key event in this disease.

Besides their role as autoantigens, nucleosomes also participate in the evolution of tissue lesions, especially glomerulonephritis. The binding of antinuclear antibodies to nucleosomes (either in the circulation or locally) can be a potential phlogistic threat for the glomerulus. When we analyzed the "cross-reactivity" of anti-DNA antibodies with heparan sulfate, we discovered that this binding to heparan sulfate was not a direct binding but was mediated by nucleosomes [17]. Because of the pivotal role of heparan sulfate for the charge-dependent permeability of the GBM [61], and the fact that neutralization of, or antibody binding to, heparan sulfate leads to massive albuminuria [62], this autoantibody binding to heparan sulfate might be a critical pathophysiologic event. Thus the important question arose: Do histones and DNA or, physiologically more relevant, nucleosomes mediate the binding to heparan sulfate in the GBM in vivo? Indeed, we and others have proved this to be the case both for histones/DNA [63, 64] and for nucleosomes [21], as analyzed in an in-vivo renal perfusion system in the rat. Perfusion of non-complexed, purified antinuclear autoantibodies did not lead to glomerular binding, thereby refuting the previously suggested possibility of "direct" binding to GBM constituents [9, 15, 65]. Removal of heparan sulfate from the GBM (by prior perfusion with heparan sulfate-degrading enzymes) considerably reduced GBM binding of nucleosome-complexed autoantibodies but did not completely prevent it [21]. Recent findings have indicated that collagen IV also can act as a ligand for such nucleosome-complexed autoantibodies [66, 67]. It is interesting that the presence of nucleosome-complexed autoantibodies showed a high correlation with the occurrence of lupus nephritis in both experimental lupus in mice [68] and patients [69]. Because heparin has strong structural similarities with heparan sulfate, we evaluated whether heparin could inhibit the binding of nucleosome-complexed autoantibodies to the GBM. In the in-vivo renal perfusion system, heparin completely prevented this binding. Moreover, long-term administration of heparin and non-coagulant heparin analogues in MRL/lpr mice prevented immune-complex deposition in the glomerular capillary loops, reduced the severity of the glomerulonephritis, and prevented albuminuria [70].

What is the evidence that this nucleosome-mediated binding occurs in lupus nephritis? (*1*) Analysis of GBM heparan sulfate staining with immunofluorescence, using GBM-heparan sulfate-specific monoclonal antibodies [62, 71], revealed a strong reduction of GBM-heparan sulfate staining both in human [72] and in murine lupus nephritis [73]. This lack of staining was not due to a reduction of the amount of heparan sulfate, but to masking of the heparan sulfate by nucleosome-containing immune complexes [73]. (*2*) Using nucleosome-specific monoclonal antibodies as a probe, we recently identified for the first time nucleosomes in glomerular immune deposits in 45% of the biopsy specimens from patients with diffuse proliferative (WHO class IV) lupus nephritis [74]. The presence of histones in 100% of these biopsies confirmed previous reports [75]. The difference between the frequency of positive staining for nucleosomes and histones in the deposits in these biopsies is in all likelihood due to the fact that nucleosome-specific epitopes, in contrast to histone-specific epitopes, are masked by autoantibodies bound to these epitopes in vivo. Although the biopsy specimen of the patient presented today could not be evaluated, a representative example from another patient with WHO class IV lupus nephritis shows the presence of nucleosomes and histones (Fig. 4). (*3*) Nucleosome-specific antibodies can be eluted from glomeruli [56, 76]. In summary, it appears that nucleosomes contribute importantly to the development of lupus nephritis by targeting antinuclear antibodies to the GBM.

Anti-C1q autoantibodies. In recent years it was found that anti-C1q autoantibodies were associated with lupus nephritis. In the 1970s, much attention was given to the measurement of circulating immune complexes in various diseases [77]. Although widely employed in those years, these measurements have not become a clinically useful tool for the diagnosis and management of patients with autoimmune diseases. One of the methods used to measure immune complexes was based on the interaction of these complexes with the complement component C1q. But not only high-molecular-weight material (immune complexes) interact with C1q, but also low-molecular-weight 7S material [78]. This "low-molecular-weight" 7S material was identified as antibodies reacting via their antigen-binding site, and not via their Fc part, with the collagen-like region of C1q. Although these antibodies have been found predominantly in patients with SLE [79], the presence of IgG anti-C1q is not restricted to patients with SLE; it is also found in patients with mixed connective tissue disease, ankylosing spondylitis, polyarteritis nodosa, mixed cryoglobulinemia, membranoproliferative glomerulonephritis, anti-GBM nephritis [80], and in older healthy controls [81].

In a retrospective analysis of SLE patients, Siegert et al found correlations between the titers of anti-C1q antibodies and the occurrence of nephritis, hypocomplementemia, and titers of anti-dsDNA antibodies [82]. This group confirmed the correlation with nephritis in a prospective analysis; moreover, an increase in serum anti-C1q antibodies was followed by the development of a proliferative glomerulonephritis [83]. In the patient presented today, we retrospectively measured the level of anti-C1q antibodies in the blood samples obtained during the fourth renal flare (Fig. 1) according to previously described methods [84]. As Figure 2



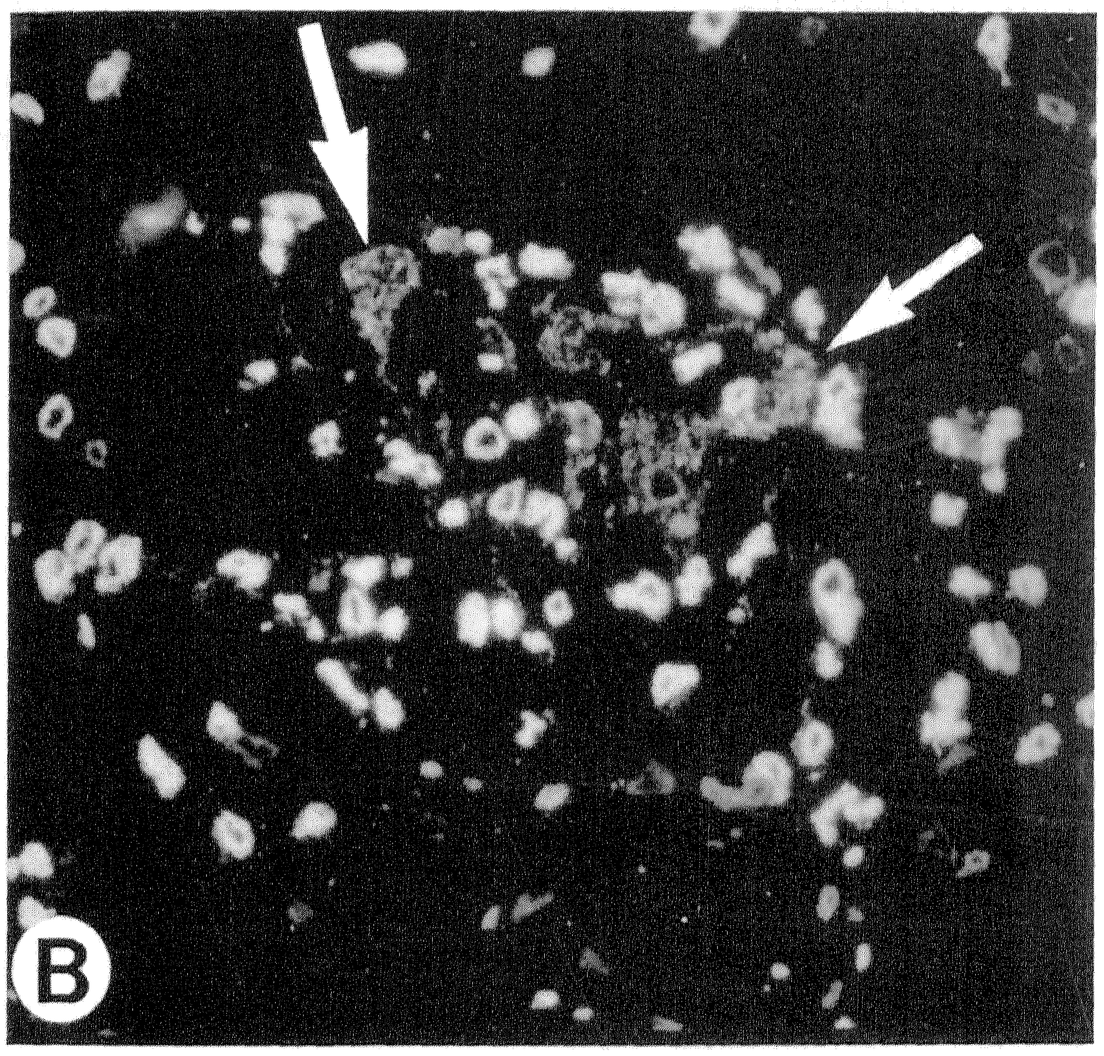


Fig. 4. Representative examples of immunofluorescence microscopy of a renal biopsy of a patient with diffuse proliferative lupus glomerulonephritis (WHO class IV). *A* Staining with a polyclonal rabbit anti-H3 1–21 antiserum (kindly provided by Dr. Sylviane Muller, CRNS Strassbourg, France). The staining reveals abundant histone deposits in the walls of the capillary loops (× 300). *B* Staining obtained with a mouse nucleosome-specific monoclonal antibody LG10–1 (generously provided by Dr. Marc Monestier, Temple University, Philadelphia, Pennsylvania). In addition to prominent anti-nuclear staining, nucleosome deposits are seen segmentally in the capillary loops (*indicated by arrows*) (× 200).

shows, we indeed noted a significant rise in the anti-C1q antibody titer during her last renal exacerbation. Recently, a prospective study investigated whether increases in anti-C1q antibody titers could better predict renal manifestations in SLE than could rises in anti-DNA titers [85]. This study was carried out in the same patients in whom Kallenberg's group established that measurements of anti-dsDNA titers can predict lupus disease flares [6]. Increases in anti-C1q levels were associated with the occurrence of biopsy-proven proliferative glomerulonephritis (WHO class III or IV), while increases in anti-dsDNA levels preceded all (renal and non-renal) disease exacerbations in SLE. Increases in anti-C1q coincided with increases in anti-dsDNA titers. The mean time period between the occurrence of a significant increase in anti-C1q or anti-dsDNA and the renal relapse was 2.3 months for both autoantibody specificities. These observations led the authors to suggest that serial measurements of anti-C1q levels might be useful in predicting a renal relapse of proliferative lupus nephritis [85]. An intriguing question is whether these antibodies have a pathogenic role in the development of the proliferative glomerular lesions. In the proliferative forms of SLE glomerulonephritis, C1q is abundantly present in the immune deposits. One could envisage that this tissue-bound C1q can serve as a planted antigen for anti-C1q binding [86], especially because these anti-C1q antibodies do not bind to fluid-phase C1q but only to C1q in the solid phase [84]. In this way, anti-C1q antibodies could amplify an ongoing glomerular inflammation.

Treatment options in lupus nephritis

There is no definitive cure for SLE and thus none for lupus nephritis. Therefore, the major aim of treatment is to reduce the symptoms and halt progression of the disease. Because many of the drugs currently used have considerable side effects, the choice of treatment should be based on balancing the benefits and risks. Proper assessment of the pros and cons requires prospective randomized trials showing the efficacy and side effects of the various therapeutic options. This starting point, however, immediately poses a problem: only a few such studies are available, those mainly carried out at the U.S. National Institutes of Health (NIH). Also needed is an appropriate measure of disease activity, especially because the course of SLE is so unpredictable. Ideally, this measure would monitor overall disease activity. Several scoring systems have become available [87, 88], but few of these have been used longitudinally. Therefore, most studies on the treatment of lupus nephritis do not provide data on the overall disease course and focus on "renal" end points [doubling of serum creatinine or development of end-stage renal failure (ESRF)].

Various studies on the prevalence of ESRF in lupus make clear that the great majority of patients who progress to ESRF have either a WHO class-III or class-IV glomerulonephritis. We are therefore justified, I believe, in reserving the more aggressive treatment modalities for patients with these proliferative forms of lupus nephritis. No controlled trials have evaluated treatment of other forms of lupus nephritis (mesangial, class II or membranous, class V), so treatment recommendations for the latter forms are based on empiric grounds and open studies. In general, treatment with corticosteroid monotherapy in mesangial lupus nephritis (WHO class II) produces satisfactory results [2]. Some authors even advocate not treating the renal lesions, but directing therapy at the extrarenal manifestations [89, 90]. In any case, one should be aware of the possibility that the lesion can progress to a proliferative form [89]. This transformation generally is heralded by nephritic urinary sediment and an increase in proteinuria.

We currently treat SLE patients who have a pure membranous glomerulonephritis with a combination of corticosteroids and azathioprine (AZA). However, Sloan et al recently found, in a retrospective analysis, that SLE patients with a pure membranous glomerulonephritis (WHO class V.A/V.B) who were treated with

prednisone monotherapy had an excellent renal survival. In contrast to this, patients with membranous lupus nephritis accompanied by proliferative lesions (the former WHO class V.C/V.D; see Table 1) had a much worse prognosis, even though most of these patients also had received cyclophosphamide (CPM) or AZA [91]. This study clearly indicates that the course of membranous glomerulonephritis formerly classified as WHO class V.C/V.D differs from that of membranous glomerulonephritis classified as WHO class V.A/V.B. If renal function is impaired or deteriorates in a patient with pure membranous lupus glomerulonephritis, one could consider treatment with oral CPM to preserve renal function, as suggested for idiopathic membranous glomerulonephritis [92]. In contrast to this, monthly intravenous CPM pulses did not preserve renal function in prospective randomized studies in patients with idiopathic membranous glomerulonephritis [93, 94]. Unfortunately, no such controlled studies are available on membranous lupus nephritis, so we do not know whether CPM should be used for the treatment of lupus membranous glomerulonephritis. If CPM is prescribed, the experience in idiopathic membranous glomerulonephritis suggests that continuous oral administration is preferable over monthly intravenous pulses. Because of the lack of data on long-term effects of CPM treatment in lupus membranous glomerulonephritis, Appel and Valeri suggested that if treatment with CPM is instituted, the treatment should be evaluated after six months; if treatment is unsuccessful, the drug should be discontinued [90]. The experience with cyclosporine A (CsA) in membranous lupus nephritis is limited. Although CsA reduced proteinuria in all patients in one study, in 4 of 10 patients proteinuria increased again after cessation; more worrisome, glomerular lesions progressed in all 5 patients rebiopsied [95]. Furthermore, CsA induced hypertension and nephrotoxicity in one-half of patients, was associated with disease flares after drug cessation, and did not influence autoantibody production [96]. Although obtained in a small number of patients, these results suggest that CsA is not a reasonable therapeutic option in SLE.

The occurrence of a pure interstitial nephritis in SLE is rare, and the literature contains no therapeutic guidelines for it. We can reasonably assume that this condition should be treated like other forms of non-SLE acute interstitial nephritis. That is, therapy could comprise oral prednisone monotherapy, in severe cases preceded by a short course of intravenous methylprednisolone [97, 98].

For advanced glomerulosclerosis in lupus (WHO class VI), intensification of immunosuppression confers no benefits. Treatment for this group of patients focuses on the extrarenal lupus manifestations and on alleviating the consequences of chronic renal insufficiency (protein restriction, antihypertensive treatment, oral phosphate binders, vitamin D analogues, and erythropoietin substitution). With the good results that renal replacement therapy offers patients with SLE, we should not expose patients with irreversible renal lesions further to the risk of immunosuppression (until the time of transplant).

Cameron analyzed the five-year patient survival of patients with WHO class IV glomerulonephritis over the last 40 years and noted a steep increase in the early 1970s, from about 25% in the decade before to 76%–78% [99]. This improved outlook undoubtedly is due to the introduction of cytotoxic drugs (mainly AZA and CPM) into the therapeutic armamentarium and to the increased availability of dialysis to treat ESRF. A meta-analysis by Felson and Anderson made it clear that the addition of cytotoxic immunosuppressive drugs (AZA or CPM) to prednisone improved renal survival in comparison to treatment with prednisone alone [100]. The histologic analysis by Balow and colleagues explained this beneficial effect [101]. In repeat biopsies of SLE patients treated with prednisone monotherapy, the chronicity index increased on average 1.4 points/year; in the AZA- or CPM-treated patients, this index remained stable over time. The same investigators were the first to report that a chronicity index of more than 3 (on a total score of 12) strongly predicted ESRF [102]. These observations raise the question: which of the two cytotoxic drugs, AZA or CPM, is superior? Clinical trials from the NIH showed that drug regimens containing either intravenous or oral CPM were superior to prednisone alone in terms of preventing ESRF [103, 104]. But AZA, combined with low oral doses of prednisone, did not significantly differ from CPM-containing regimens or from prednisone monotherapy. Further, the NIH studies showed that a regimen of monthly intravenous pulses of CPM was comparable to continuously used oral CPM in terms of efficacy, but was less toxic [103]. Although desperately needed, no further controlled trials have compared CPM and AZA in terms of efficacy and toxicity.

Cameron [99] tried to overcome this lack of information by comparing both the renal and patient survival of patients with diffuse proliferative lupus nephritis treated with either CPM [103] or AZA [99, 103, 105–107] and found no differences in either renal or patient survival. Cameron's comparison conforms with the results of the NIH studies, which also found no differences between CPM and AZA [103, 104]. The superiority of CPM over AZA thus has not been proven. This conclusion is important in view of the severity and higher frequency of side effects of CPM. Treatment with CPM is associated with amenorrhea in 50% to 70% of patients after long-term oral use and in 30% after intravenous administration of CPM pulses [108]. In patients given intravenous pulses of CPM, the incidence of amenorrhea increases with the number of pulses and the patient's age [108]. The fear of this side effect caused the patient presented today to refuse treatment with CPM on three occasions. Data on the effect of CPM on spermatogenesis are curiously lacking. One report about CPM therapy for nephrotic syndrome due to minimal-change disease suggests a threshold of 12 to 17 g as the cumulative dose above which oligospermia can occur [109]. These data contrast sharply with the effects of AZA on gonadal function. Experience gained from renal transplant patients indicates that AZA does not affect reproductive ability both in men and women. Even pregnancies during which AZA is administered are relatively safe, showing only a slight increase in congenital malformations [110]. In contrast, giving CPM during pregnancy is absolutely contraindicated because of its strong teratogenic potential. Uro-epithelial toxicity, ranging from hemorrhagic cystitis to invasive bladder carcinoma, is also a unique side effect of CPM. This complication occurs more frequently after oral than after intravenous administration of CPM and is related to the total cumulative dose [103, 111]. Treatment with long-term oral CPM is associated with the development of hemorrhagic cystitis in 17% of lupus patients [103] and in 43% of patients with Wegener's disease [112]. In this latter group of patients, 2.8% developed bladder cancer, which represented a 33-fold increase in the risk for this complication. An increased frequency of other malignancies, mainly non-Hodgkin's lymphoma (relative risk: 10.9) and skin cancers (relative risk: 5),

is observed during treatment with AZA or CPM [113]. Although we have no good data that compare the carcinogenic potential of these drugs, we have more long-term experience with AZA.

In addition to oral prednisone, AZA, or CPM, another therapeutic modality frequently employed in the treatment of lupus nephritis is intravenous pulses of high doses of methylprednisolone (ivMP). These high dosages of steroids likely have a more rapid and a stronger effect on the inflammatory and autoimmune response than does oral prednisone [89]. A number of uncontrolled studies [reviewed in Ref. 89] and one controlled study [114] showed that ivMP in conjunction with low doses of oral prednisone promptly improved renal function and autoimmune parameters in most patients, but the effect on proteinuria took longer. These studies also suggested that the ivMP/low-dose oral prednisone regimen had higher efficacy combined with lower toxicity when compared to high oral doses of prednisone alone. None of these studies, however, established the long-term effect of ivMP on the preservation of renal function. The histologic followup study by Balow et al [101], which evaluated oral prednisone treatment, raises the question whether treatment with steroids alone can prevent the progression of chronic lesions. Indeed, a prospective randomized study from the same group compared six monthly ivMP pulses with intravenous pulses of CPM administered for six months (6 pulses) or 30 months (14 pulses) and found that treatment with ivMP was associated with a significantly greater risk for deterioration of renal function and a higher relapse rate than treatment with CPM pulses [115]. Thus ivMP alone, without cytotoxic drugs, constitutes insufficient treatment.

What conclusions can we draw from these studies? First, it has not been proven that AZA is less effective than CPM, but it is certainly less toxic. Second, in the initial treatment of lupus nephritis, ivMP is as good as high dosages of oral prednisone (1.0 mg/kg) but has fewer side effects. Therefore, a combination of AZA (which prevents progression of chronic lesions) and an induction treatment with ivMP (which rapidly induces regression of the acute lesions) might be an ideal combination, combining efficacy with less toxicity. A recent report from the Academic Medical Center of the University of Amsterdam showed, unfortunately in an uncontrolled study, that this combination therapy is effective and associated with little toxicity [116]. Thirteen patients with recent-onset (median duration, four months) diffuse proliferative lupus nephritis (mean serum creatinine, 109 μmol/liter (1.2 mg/dl); GFR, 58 ml/min) were treated with three courses of 1 g ivMP given three times (2 and 4 weeks apart), 20 mg oral prednisone, and 2 mg/kg AZA. This regimen improved renal function in all patients (mean serum creatinine, 68 μmol/liter (0.7 mg/dl); GFR, 102 ml/min at mean followup of 7 years), reduced proteinuria (only four had 1.0–1.5 g/24 hr at last followup), and improved serologic parameters. Side effects occurred in two patients: re-entry tachycardia and septicemia. Although herpes zoster developed in one patient, no other major bacterial, viral, or opportunistic infections occurred. The promising results of this uncontrolled study prompted the Dutch Working Party on SLE to embark recently on a national, prospective randomized trial comparing intravenous pulses of CPM (6 monthly pulses, and quarterly pulses for the following 18 months) and high oral doses of prednisone (1 mg/kg) with the combination therapy of ivMP, prednisone, and AZA [as described in Ref. 117]. The results of this study should provide more definitive conclusions regarding the efficacy and side effects of CPM/prednisone versus AZA/prednisone treatment.

Finally, let me say a few words about the therapeutic potential of plasmapheresis in lupus nephritis. Although two groups claimed positive short-term effects in uncontrolled studies [118, 119], subsequent randomized trials failed to confirm their enthusiasm [120, 121]. A large-scale, prospective randomized trial provided a definitive answer regarding the efficacy of plasmapheresis [122]. The addition of plasmapheresis to immunosuppressive therapy did not confer a significant benefit. Although the results of a prospective German study employing sequential plasmapheresis and intravenous CPM are not yet available [123], it seems justifiable to conclude on the basis of current information [124], that plasmapheresis is not a useful adjunct to immunosuppressive therapy in patients with lupus nephritis.

Intensification of immunosuppressive treatment is generally indicated if a renal flare develops. The identification of such an exacerbation usually is based on changes in clinical parameters, such as an increase in hematuria or proteinuria, or a deterioration of renal function. The observation that 89% of renal and nonrenal disease flares occur 10 weeks after a significant rise of the anti-DNA titer [6] led Kallenberg's group to investigate whether these relapses can be prevented by increasing the prednisone dose, irrespective of clinical symptoms, if such a rise in anti-dsDNA occurs [125]. Anti-dsDNA titers were measured monthly with the Farr assay. If the anti-DNA titer rose more than 25%, patients were randomly assigned to either continuation of their current medication or to a 30 mg increase of their current prednisone dose. The titer rose significantly in 46 of 156 patients; 24 were randomized for continuation of their existing medication, and 22 patients were given an increased prednisone dose. The relapse rate was higher in the group with unchanged medication than in the prednisone group (20 versus 2, $P < 0.001$), but a significant difference was noted ($P < 0.001$) only for minor relapses and not for major relapses ($P = 0.12$). Renal relapses occurred five times in the conventional treatment group and two times in the prednisone group; this difference was not significant. Furthermore, this approach did not lead to a difference in the cumulative or mean daily dose of prednisone after randomization. Therefore one has to conclude that this serologically guided treatment protocol cannot prevent renal relapses. It remains to be determined whether the use of a different serologic marker (possibly nucleosome-specific antibodies or anti-C1q antibodies) can yield more favorable results.

Renal replacement therapy in SLE

Progression to end-stage renal failure. The patient presented today developed ESRF within four years. Nossent's recent review of the literature indicates that this complication develops in approximately 20% of the patients with lupus nephritis (Table 2) [126]. These data suggest that the incidence of ESRF has been relatively stable over the last three decades. In most studies, the mean time between onset of lupus nephritis and ESRF is five years [127, 128]. Nossent and colleagues in cooperation with the Dutch Working Party on SLE studied 55 SLE patients who developed ESRF [128]. These patients had at least one, and sometimes more, renal flares after the onset of nephritis, as did the patient presented here. The standardized SLE Disease Activity Index (SLEDAI) [129] applied at three-month intervals revealed that in the period preceding ESRF, 15% of the scores were

Table 2. Percentage of SLE patients with glomerulonephritis developing end-stage renal failure (ESRF) during the last four decades[a]

Time period	Number of patients with lupus nephritis	Number of SLE patients with ESRF	Percentage
1950–1960	54	26	48.1
1961–1970	301	40	13.3
1971–1980	601	145	24.1
1981–1992	317	58	18.3
All periods	1273	269	21.1

[a] Recalculated from data reviewed in Ref. 126.

Table 3. Factors associated with an increased risk for the development of end-stage renal failure in SLE

Factor	Reference
Elevated initial serum creatinine (in most studies ≥ 100 μmol/liter, 1.1 mg/dl)	[91, 105, 133, 140–144]
Nephrotic-range proteinuria	[105, 144, 145]
Low C3	[105, 143, 146]
Hematocrit ≤ 26%	[143, 146]
Hypertension	[143]
WHO class IV	[102, 105, 133, 140, 147]
Activity index ≥ 12	[102, 140, 142, 143]
Chronicity index ≥ 3	[102, 105, 140, 142, 143]
No normalization of elevated serum creatinine at 48 weeks	[141]
Treatment with prednisone only	[100, 104, 148]
Persistent disease activity	[105, 126]
Male gender	[128, 135, 140]
Black race	[143, 146, 149, 150]
Age ≤ 24 years	[105, 140]
Low socioeconomic status	[149]

high (≥ 10) and 51% were moderately high. The regression line of all SLEDAI scores over time prior to dialysis showed a slow but significant rise. These observations indicate that, in general, this group of patients had active disease before reaching ESRF. Furthermore, in the period before reaching ESRF, disease quiescence is exceptionally rare in lupus patients progressing to renal failure [126]. Therefore, as one would expect, persistent or remittent disease activity is a major risk factor for ESRF.

The literature cites a number of other factors that increase the risk for ESRF in patients with lupus nephritis (Table 3). Not all these parameters have been confirmed in all studies, probably because of differences in definitions used, composition of the cohort studied, duration of the followup, and the number of patients included. Many of these risk factors were present in today's patient: hypertension, nephrotic-range proteinuria, low C3, WHO class IV, activity index ≥ 12, remittent disease activity, and age ≤ 24 years).

Dialysis. Hemodialysis initially was not offered to lupus nephritis patients because of the systemic nature of their illness. But in the early 1970s, it became clear that these patients tolerated hemodialysis treatment as well as did patients with non-SLE renal diseases [130]. Survival rates for patients with lupus were initially lower in some [131], but not all studies [132], however. This increased mortality rate was mainly due to infections secondary to continued immunosuppressive treatment. At present, the five-year survival rate of SLE patients treated with dialysis is at least as good as that for the non-lupus population, and ranges between

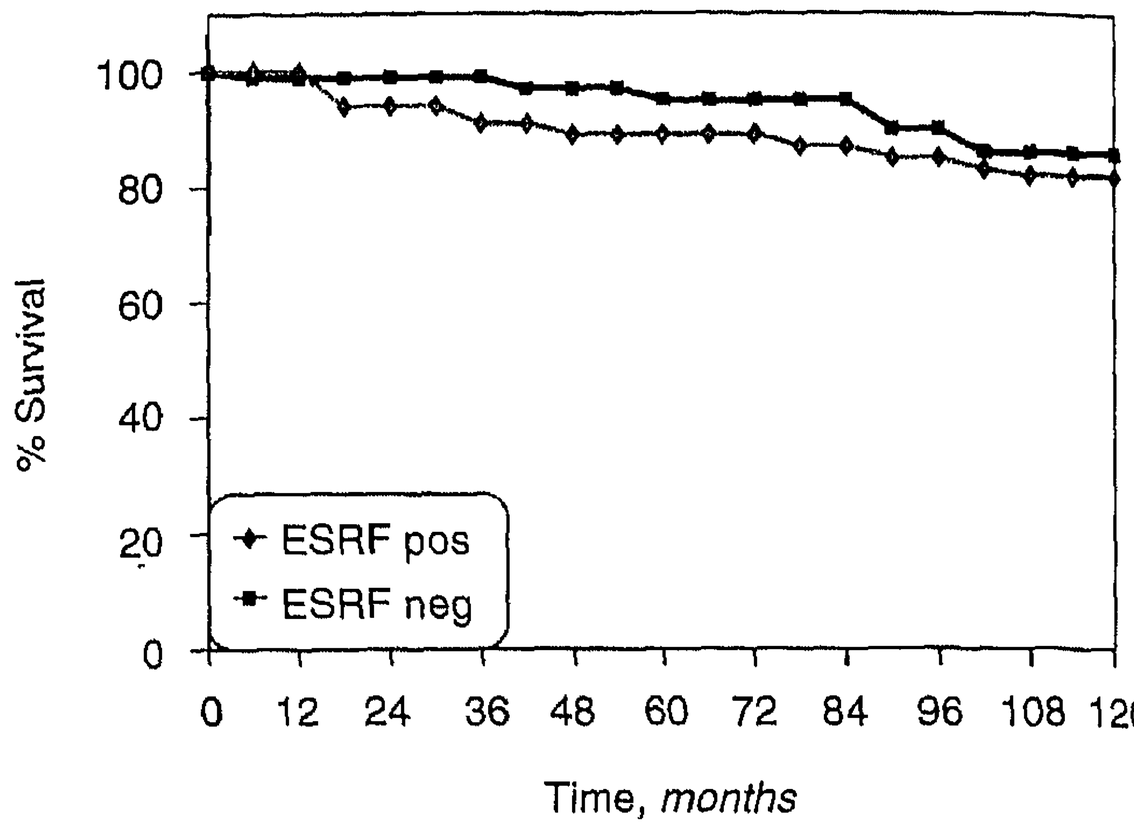


Fig. 5. Actuarial patient survival of 55 SLE patients who progressed to end-stage renal failure (ESRF pos) compared with that of 92 SLE patients who did not develop end-stage renal failure (ESRF neg). The survival curves were not significantly different. Time point zero represents the onset of lupus nephritis. (Reproduced with permission from Ref. 126.)

80% and 90% [127, 128]. Because the prognosis during dialysis treatment is relatively so good in patients with SLE, it is no longer necessary to prevent ESRF at all costs in patients with already impaired renal function. Although intensification of immunosuppression decreased the cumulative incidence of ESRF in some studies, it did not improve patient survival rates [104, 133]. Actually, in the lupus nephritis studies by the Dutch Working Party on SLE, patient survival calculated from the onset of lupus nephritis was similar for patients with lupus nephritis who did (n = 55) or did not (n = 92) reach ESRF (Fig. 5) [126]. These data clearly show that dialysis is not associated with a higher mortality when compared to aggressive therapy of far advanced lupus.

What is the influence of dialysis on lupus disease activity? Before addressing this question, we should note that sometimes it is difficult to determine whether a certain condition (for instance, pericarditis) is an ESRF-related manifestation or an expression of lupus disease activity. Given this limitation, we can say that disease activity generally decreases during dialysis, although disease manifestations can persist or even develop, especially in the first year after the start of dialysis [126]. Nossent and colleagues compared in 55 SLE patients undergoing dialysis treatment—either hemodialysis (n = 32) or CAPD (n = 23)—the maximal non-renal SLEDAI scores before and after the start of dialysis, and found that this score decreased significantly ($P < 0.001$) after dialysis was started [128]. In 49% of the patients, maximal non-renal disease activity decreased, in 42% remained stable, and only in 9% showed progression [128]. No differences were found between patients treated with hemodialysis or CAPD. The SLEDAI is the most sensitive index for measuring changes in SLE disease activity over time [134]. Nossent et al used another estimate of disease activity, the time-adjusted event rate of specific disease manifestations, and discerned no significant differences in disease activity between the periods before and after the start of dialysis, although the events during dialysis occurred during minimized immunosuppression [128]. For instance, azathioprine or cyclophosphamide administration was stopped in

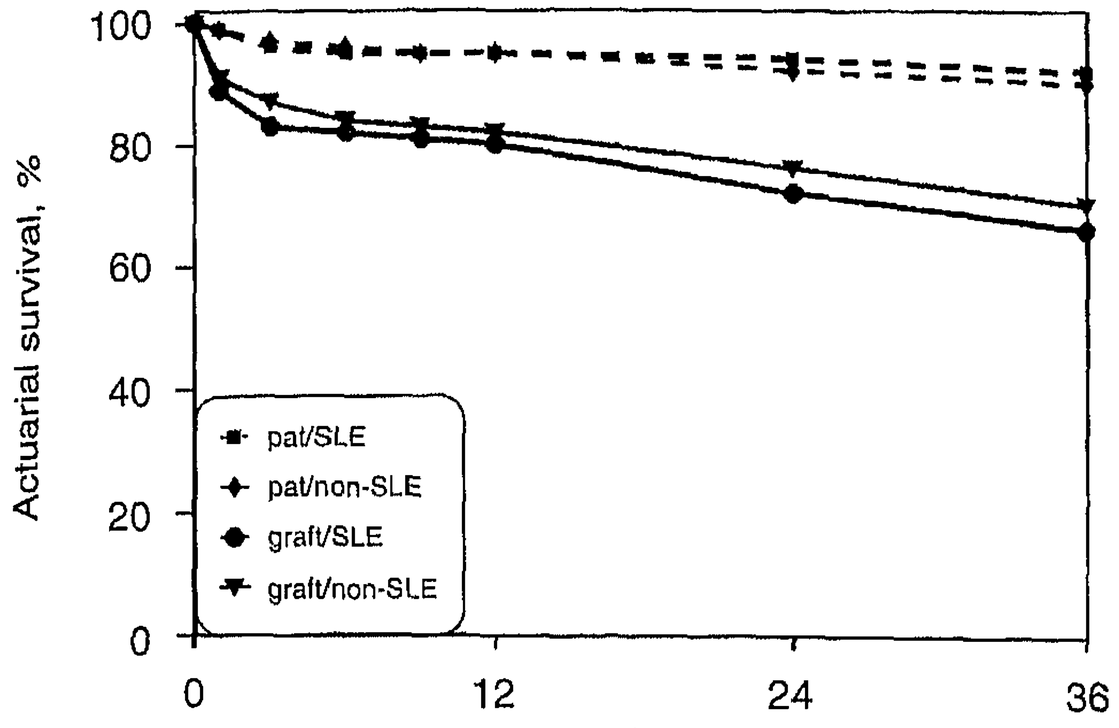


Fig. 6. Graft (solid lines) and patient (dashed lines) survival after renal transplantation in SLE patients (n = 165) compared with patients with non-SLE glomerular diseases (n = 21726); 1984–1992. Only first transplants with a cadaveric donor kidney were used for this analysis. Both the graft and patient survival were not significantly different between the two groups. (Data were generously provided by Dr. Guido Persijn, Eurotransplant, Leyden, the Netherlands.)

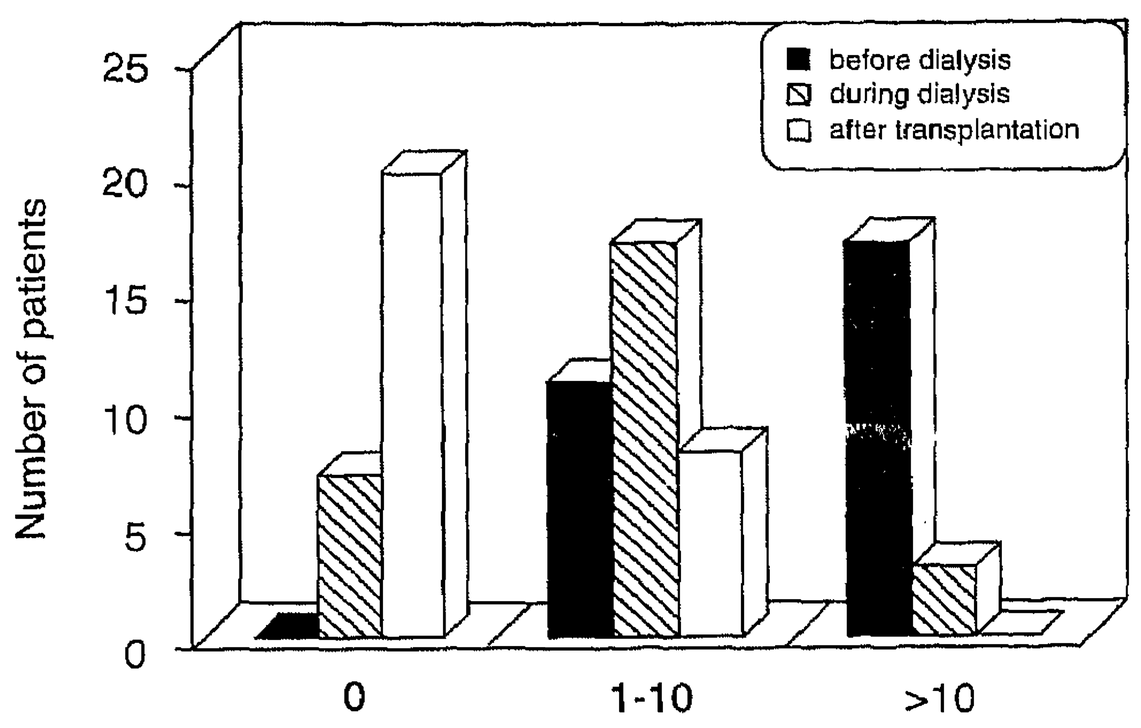


Fig. 7. Lupus disease activity in 28 SLE patients who received a renal allograft. Disease activity was assessed by the maximal non-renal SLEDAI before (■) and during (▨) dialysis and after renal transplantation (□). The SLEDAI scores were divided in three groups: 0, no disease activity; 1–10, moderate disease activity; and >10, high disease activity. Note that prior to dialysis none of these patients had absent disease activity, but after renal transplantation none of the patients had high disease activity. (Figure drawn from data published in Ref. 135.)

90% of the patients, and the prednisone dose was considerably reduced in 55%.

Renal transplantation. The patient presented in this Forum did very well after renal transplantation. She had only a borderline acute graft rejection three months after transplantation and no major lupus manifestations during a followup of 4 years. Is her course a fair general representation of all lupus patients after renal transplantation? In our Dutch survey on renal transplantation in lupus patients, both the one- and three-year graft survival was 68%, and patient survival was 87% [135]. Graft survival was somewhat lower than that generally achieved nowadays. However, patient data were collected over an eight-year period, from 1980 to 1988, and one-half of the patients received transplants before the routine use of cyclosporine A as a basic immunosuppressive drug. More recent data obtained from Eurotransplant show that graft and patient survival rates for SLE patients after renal transplantation are not significantly different from those for patients with non-SLE glomerular diseases (Fig. 6). Similar results have been reported in the literature [136–138].

What happens to SLE disease activity after renal transplantation? In general, disease activity declines further. In the Dutch analysis, the majority of patients (72%) had no disease activity after renal transplantation, as judged by SLEDAI scores every three months during a median followup of 31.5 months (range, 3–89 months) [135]. Figure 7 compares disease activity (using maximal non-renal SLEDAI scores) before dialysis, during dialysis, and after renal transplantation in SLE patients. It is interesting that high lupus disease activity before or after the start of dialysis, a duration of dialysis of less than 12 months, or a high antilymphocyte reactivity before transplantation did not adversely affect graft survival. In only one patient was a recurrence of the original disease diagnosed. In a recent survey of the literature, 5 of 321 patients showed recurrence of lupus nephritis after renal transplantation [126]. If this total number is corrected for the patients who lose their grafts within the first year, the calculated cumulative incidence of recurrent disease is 1.9%. This experience contrasts with a report from Nyberg and colleagues, who found signs of recurrence in electively taken transplant biopsy specimens in 7 of 16 SLE patients (43%) [139]. The choice of the parameters they used to define recurrence—namely, mesangial proliferation and deposition of IgM and C3—is debatable, because these features are not specific for lupus nephritis, especially in transplant biopsies. Thus, most of the literature suggests that in general, lupus patients have a reduction of disease manifestations and a very low risk of recurrence after transplantation. It remains intriguing why these patients respond so well after transplantation, since they are usually treated with the same kind of drugs that failed to control their disease before transplantation. In summary, dialysis and renal transplantation are good treatment modalities for SLE patients with ESRF. During renal replacement therapy, lupus disease activity generally subsides, and the likelihood of recurrence of lupus nephritis after renal transplantation is low.

QUESTIONS AND ANSWERS

DR. NICOLAS E. MADIAS (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): What do we know about the nature of the nucleosomal epitopes that appear to drive the autoimmune response in systemic lupus erythematosus?

DR. BERDEN: This is an important question. Since it is difficult to analyze these epitopes with polyclonal auto-antibodies, we and others have tried to circumvent this problem by analyzing the antigen specificity of different nucleosome-specific monoclonal antibodies [151–154]. These analyses revealed that H2A-H2B/DNA seems to harbor the major epitopes and, to a lesser extent, also H3-H4/DNA. Time studies, mainly in lupus mice, indicate that antibodies to H2A-H2B/DNA are formed earlier in the disease than are those against H3-H4/DNA [26, 55]. We also tested these monoclonal antibodies on 53 partly overlapping synthetic peptides, covering all histone proteins, and found with

one exception (peptide 83–100 from histone H3) no or only very low reactivity; thus, linear epitopes probably are not very important [60, 151]. Because some of these monoclonal antibodies react better with the total nucleosome than with subnucleosomal structures, additional epitopes might exist that are still undiscovered [151]. These data confirm previous observations that nucleosome-specific antibodies recognize conformational epitopes created by the interaction between DNA and histones. Relevant epitopes might not be expressed by native nucleosomes but are only presented on altered nucleosomes. These alterations (protein modification/fragmentation) can be induced by oxygen radicals produced during aberrant apoptosis [47, 48]. These novel epitopes, not expressed on native nucleosomes, are then recognized by T-cells. At the moment we are probing these possibilities by using nucleosomes released from normal and lupus-derived apoptotic cells as antigenic targets. This investigation might elucidate the driving epitope within the nucleosome. Recently Datta's group localized three regions in the core histones 10–33 H2B, 16–39 H4, and 71–94 H4 that acted as epitopes for pathogenic T-helper cells [155].

Dr. Madias: As you know, Madaio and coworkers have isolated a number of monoclonal antibodies with anti-DNA activity from various murine models of SLE [15]. These antibodies exhibit differences in their level of nephritogenicity. Further, individual nephritogenic antibodies consistently display a distinct pattern of glomerular injury. Could you please reconcile these observations with the nucleosomal theory?

Dr. Berden: We think that these observations are due to the fact that these monoclonal antibodies are complexed to a variable degree to nucleosomes, depending on the specificity of the monoclonal antibody and the hybridoma culture conditions. As we have shown, it is sometimes very difficult to remove all the complexed nucleosomal material [21]. After purifying anti-nuclear monoclonal antibodies and carefully checking for remaining nucleosomal impurities (with ^{32}P radiolabeling to detect DNA and agarose gel electrophoresis with double silver staining to detect histones), we never found glomerular localization with purified antibodies [21]. Also, cross-reactive binding of monoclonal anti-nuclear antibodies to other non-DNA antigens like heparan sulfate was abrogated by this purification [17]. The observation from Dr. Madaio's group that such a cross-reactivity [14] is a distinguishing feature of their nephritogenic monoclonal antibodies [156, 157] strongly, albeit indirectly, indicates that complex formation with nucleosomes is instrumental for induction of glomerular deposits.

Another line of evidence indicates that Madaio's monoclonal antibody preparations are complexed to nucleosomes. These investigators reported that several of their monoclonal antibodies are able to penetrate cells and to localize into the nucleus [158]. We [159] and others [160] have shown recently that this intracellular uptake of autoantibodies critically depends on the presence of nucleosomal material bound to the antibody, since purified antibodies did not bind and were not internalized. In our hands, the observed nuclear localization was a fixation artifact; if we prepared the kidney with perfusion-fixation with PLP (periodate lysine-paraformaldehyde) instead of using tissue fixation with acetone, we found not nuclear but cytoplasmic localization, similar to that observed with confocal laser microscopy in living cells [159]. Although this in-vivo ANA phenomenon is artifactual, we agree with Dr. Madaio and his coworkers that the induction of glomerular deposits is characteristic for pathogenic and nephritogenic autoantibodies, because it is displayed by autoantibodies complexed to nucleosomes. These same nucleosome-complexed autoantibodies can bind to the GBM in vivo [21] and are related to the onset [161] and exacerbation [162] of lupus nephritis in patients with SLE. Also, Ohnishi and colleagues concluded that pathogenic anti-DNA antibodies can: (*1*) bind to the GBM via histone/DNA (nucleosomes), and (2) display an in-vivo ANA phenomenon after acetone tissue fixation [163].

So, to summarize a long answer, both our and Dr. Madaio's results are consistent with the fact that certain anti-nuclear antibodies can induce glomerular deposits, but our explanations for this glomerular binding differ. They attribute their observations to direct cross-reactive binding of monoclonal antibodies to the GBM and cell-surface constituents; we have convincing evidence that these reactivities are mediated by nucleosomes.

Dr. Behrooz Broumand (*Professor of Medicine, University of Medical Sciences of Iran, Teheran, Iran*): I have three questions. First, after transplantation most patients with SLE are treated with prednisone, azathioprine, and cyclosporine. Moreover, after renal transplantation, disease activity generally declines. Maybe it would be wise to formally study these drugs to evaluate whether they are better than either prednisone and cyclophosphamide or prednisone and azathioprine for the treatment of lupus nephritis. Second, do you think that ACE inhibitors could be used in the treatment of lupus nephritis? Besides decreasing blood pressure, they could reduce proteinuria and prevent further tubulo-interstitial damage. Finally, do you see any future for gene therapy—for instance, manipulating Bcl-2 or BAX expression in order to influence apoptosis?

Dr. Berden: To answer your first question, only limited experience, in uncontrolled studies, with cyclosporine in the treatment of class-IV or class-V lupus nephritis has been reported, and the results are not very convincing [164, 165]. What worries me about the use of cyclosporine for the treatment of lupus nephritis is that it has no effect on autoantibody formation either in humans [164] or in spontaneous murine lupus models [166]. And as mentioned, repeat biopsies during treatment with cyclosporine showed progression of histologic abnormalities [95] despite the fact that proteinuria declined. In my opinion, there is no place for cyclosporine in the treatment of lupus nephritis. You are correct that disease activity declines after renal transplantation in the majority of patients, but improvement has already begun during dialysis and before transplantation. Thus the "burn-out" of the disease is not due to post-transplant immunosuppression, but rather originates during dialysis. There is no satisfactory explanation for this "burn-out."

Your second question relates to the use of ACE inhibitors in patients with lupus nephritis. It's certainly true that inadequate blood pressure control in patients with lupus nephritis will quicken the deterioration of renal function. I am not aware of any study in lupus patients showing that ACE inhibitors are superior in this respect to beta-blockers or calcium-entry blockers. We do know that ACE inhibitors reduce proteinuria [167] and thus could slow the progression of tubulo-interstitial damage [168]. Austin et al recently showed that, indeed, tubulo-interstitial lesions, as in other glomerular diseases [169], are strong predictors for the development of end-stage renal failure [143]. Any measure that reduces this interstitial burden is therefore beneficial.

Your last question related to gene therapy. I do not think that gene treatment to influence Bcl-2 expression is currently feasible, because the margins for this approach seem rather small. In experimental animals, transgenic overexpression of Bcl-2 lead to auto-immunity [38]; in Bcl-2 knockout mice, a dramatic involution of the immune system is observed soon after birth [170, 171]. In my opinion a more realistic approach would be a search for nucleosome-derived peptides that could "turn off" the pathogenic auto-immune response. A similar approach has been successful in a number of auto-immune models, such as adjuvant-induced arthritis [172].

DR. MARIAN KLINGER (*Professor of Renal Medicine, Department of Nephrology, University School of Medicine, Wroclaw, Poland*): Your point against cyclophosphamide was very strong, but cyclophosphamide toxicity very much depends on cumulative doses. Why not start with intravenous cyclophosphamide, administer doses of, let's say, up to 100 mg/kg, and later support the remission with azathioprine? One remark: I noticed that the prednisone dose in your prospective protocol, 1 mg/kg, is higher than the dose of 0.5 mg/kg used in the original NIH protocol, so the risk of undesired prednisone effects is greater. Finally, you did not mention in your choices of therapy so-called "synchronized plasmapheresis." Do you believe that it is worth a trial?

DR. BERDEN: I did not understand your first point. Did you propose to give cyclophosphamide at 100 mg/kg?

DR. KLINGER: I propose to start with standard intravenous cyclosphamide doses and administer it until a cumulative dose of 100 mg/kg—which is, I believe, without major long-term toxicity—is reached and later try to support the remission with azathioprine.

DR. BERDEN: The protocol that you describe with initial cyclophosphamide and subsequent azathioprine has been used by a number of groups both for lupus and non-lupus glomerular diseases [122, 173]. Your presumption is that cyclophosphamide treatment is better, but you want to reduce the side effects. I think the NIH data force us to conclude that cyclophosphamide is not superior to azathioprine. So I don't believe that a six-month induction treatment, which does not exceed the cumulative dose you mentioned, is advantageous over the combination of intravenous methylprednisolone and azathioprine. Still, we have no controlled data to support either your or my approach.

You asked about the predisone dose. The two publications from the NIH group are not very specific about the prednisone dose they use [103, 104]. The different reports use between 0.5 mg/kg and 1 mg/kg, but in general they did start with 0.5 mg/kg. However, in our protocol, we taper the dose rapidly, and our cumulative cyclophosphamide dose is lower (24 months of treatment instead of 30 months).

Your last question about synchronized plasmapheresis is very current. In 1990 the group of Euler in Kiel started a trial using synchronized plasmapheresis and cyclophosphamide. The results have not yet been published, but I recently heard that they will become available soon. Theoretically, this approach is attractive, because plasmapheresis stimulates the B-cell, which makes it more susceptible to the cytotoxic drug given subsequently. We have to wait for the results of this trial before coming to any conclusions about this approach.

DR. MADIAS: Some cases of lupus are accompanied by a thrombotic vasculopathy with glomerular and vascular thrombi, often in association with a lupus anticoagulant, the clinical picture being akin to that of thrombotic thrombocytopenic purpura. Are you of the opinion that plasmapheresis with plasma infusion should not be used even in that setting?

DR. BERDEN: Some lupus patients do have renal vascular abnormalities in conjunction with anti-phospholipid antibodies. In general no correlation exists between the presence of anti-phospholipid antibodies and the development of lupus nephritis [174, 175] but, as you said, thrombi can develop in the renal vasculature [175, 176]. No data regarding optimal treatment are available; all reports have been anecdotal. We treat these patients with oral anticoagulants. We don't treat them differently with regard to the immunosuppressive regimen unless glomerulonephritis is absent. These patients, who do not have inflammatory glomerular lesions, sometimes present with acute renal failure, often accompanied by malignant hypertension. This condition has been called the "catastrophic anti-phospholipid syndrome" [177]. Plasmapheresis combined with adequate anticoagulation is currently the treatment of choice for these patients [177].

DR. S. C. DASH (*Professor of Nephrology, All India Institute of Medicine Sciences, New Delhi, India*): Do you use a threshold value for the histologic activity and/or chronicity index above which you start treatment with cytotoxic drugs?

DR. BERDEN: We base our decision for treatment with cytotoxic immunosuppressive agents only on the World Health Organization classification (either class III or IV), although the WHO classification does correlate with the NIH scoring system. In our retrospective analysis [142], we found a mean activity and chronicity index, respectively, for class III of 4.6 and 3.2, and for class IV of 8.1 and 3.9; for class II and V, these indices were much lower. Of course, it would be nice to have the results of a study in which you could further refine your therapy based on histologic criteria like the activity and chronicity indices. But it will be very difficult to perform a study in which patients are stratified according to NIH activity and chronicity indices. It is difficult enough to carry out a trial based on the WHO classification, as evidenced by the low number of controlled studies available. The only situation in which we take the NIH score into account is if the patient has advanced sclerotic glomerular lesions and a high chronicity index. The majority of these patients are categorized as WHO class VI. We are reluctant to prescribe cytotoxic drugs in this group because we don't anticipate much of a functional benefit.

DR. MIKE VENNING (*Consultant Renal Physician, Withington Hospital, University Hospital of South Manchester, Manchester, England*): I am concerned that you might have drawn some conclusions that are not fully warranted by the evidence, specifically with reference to the size of the trials and a lack of power to support negative conclusions. From conversations a few years ago with Dr. Balow, I understand that he believes cyclophosphamide to be a better therapy than azathioprine for lupus nephritis. Further, clinical experience with patients who have responded to cyclophosphamide after having relapsed or failed to achieve control with azathioprine therapy also suggests that active lupus nephritis is better treated with cyclophosphamide. The NIH study did not have sufficient power to determine whether cyclophosphamide is better than azathioprine.

Similarly, clinical experience with chronic ambulatory peritoneal dialysis (CAPD) in lupus patients suggests that the risk of peritonitis or exit-site infection is high following immunosuppression, particularly when high doses of steroids have been used. I am

concerned that sufficient data are not available to demonstrate the equivalent effectiveness of hemodialysis, which I believe to be better in this setting, and CAPD.

DR. BERDEN: I cannot exclude the possibility that the NIH studies are flawed by a class-II error, especially because the number of patients treated with azathioprine was rather small. But studies other than the NIH investigation have shown no conclusive differences between azathioprine and cyclophosphamide in the treatment of lupus nephritis [99, 100]. Actually, in the meta-analysis by Felson and Anderson, azathioprine preserved renal function better, and was associated with a lower number of deaths due to non-renal causes, than did cyclophosphamide [100]. Indeed, although our NIH colleagues advocate cyclophosphamide therapy, their results do not prove that cyclophosphamide treatment is better.

Your second comment suggested that hemodialysis is better than CAPD in patients with lupus. Clearly, CAPD bears the intrinsic risk of peritonitis and exit-site infections, possibly due to immunosuppression. But as I said, our policy—and I think that this is true for most clinics—is to reduce immunosuppression as soon and as quickly as possible. With this policy we have not observed any difference in our comparison between hemodialysis (n = 32) and CAPD patients (n = 23) in terms of survival, lupus disease activity, and complications. The incidence of peritonitis in lupus patients did not differ significantly from that in the non-lupus population. Cheigh et al came to the same conclusion [178].

DR. VENNING: But again this might be subject to a class-II error and perhaps needs further study.

DR. BERDEN: Maybe, but to my knowledge our study is the largest study available.

DR. SÁNDOR SONKODI (*Professor of Medicine and Nephrology, First Department of Medicine, Albert Szent-Györgyi Medical University, Szeged, Hungary*): May I go back to the question of cytotoxic treatment? In your opinion, what is the value of chlorambucil in the treatment of lupus nephritis? As you know, chlorambucil has fewer side effects than cyclophosphamide.

DR. BERDEN: I don't know whether it is true that chlorambucil has fewer side effects than cyclophosphamide. Furthermore, there are no solid data available about the efficacy of this drug in the treatment of lupus nephritis [164, 179]. Although we do not have experience with chlorambucil in lupus nephritis, it has been used in our department for the treatment of idiopathic membranous glomerulonephritis [93]. In this prospective trial, in which we compared chlorambucil with cyclophosphamide given intravenously, patients did not have fewer side effects with chlorambucil than with cyclophosphamide. So I prefer cyclophosphamide over chlorambucil. But again, there are no controlled studies comparing these two drugs in lupus nephritis.

DR. MADIAS: Do you envision a role for newer drugs, such as rapamycin, mycophenolate, or FK506, in the management of lupus nephritis?

DR. BERDEN: Of the drugs you mentioned, I think mycophenolate has the best potential. It has a much more profound effect on antibody production by B-cells than does azathioprine or cyclophosphamide. If the formation of autoantibodies is instrumental for the development of the different tissue lesions, this effect might be an important advantage. In addition, mycophenolate has anti-inflammatory effects, mainly because it inhibits the recruitment of neutrophils, lymphocytes, and monocytes to the inflammatory site [180, 181]. Rapamycin and FK506 are also potentially interesting drugs, although their effects on antibody production are less well established. All three drugs have shown beneficial effects on the autoimmune disease in MRL/lpr and $(NZBxW)F_1$ lupus mice [182–184].

DR. MADIAS: How do you manage patients whose disease is resistant to conventional therapy?

DR. BERDEN: It depends on the degree of renal function and the previous treatment. If the patient has received several courses of induction treatment with immunosuppressive drugs, has poor renal function, and a renal biopsy shows extensive chronic lesions, we prepare the patient for dialysis and renal transplantation. New cases are currently enrolled in the Dutch prospective trial comparing cyclophosphamide pulses with intravenous methylprednisolone, low-dose oral prednisone, and azathioprine. If it is a first manifestation or a first flare and the patient fails to respond, we switch to the other arm of our protocol. Before we started this prospective trial, we added plasmapheresis to the treatment of resistant cases, but after the results of the plasmapheresis trial became available [122], we stopped doing so.

DR. BHARAT V. SHAH (*Head of Nephrology Section, P.D. Hinduja National Hospital, Mumbai, India*): You reserve treatment with cytotoxic drugs mainly for patients with class-III and class-IV lupus nephritis. Don't you think that a class-II nephritis can progress to class-III or -IV, and that by giving cytotoxic treatment one can prevent this progression?

DR. BERDEN: You are right that transition from class-II to class-III or -IV has been documented [4]. However, this transition is generally accompanied by a worsening of clinical signs and symptoms—either the appearance of a nephritic sediment, an increase in or reappearance of proteinuria, deterioration of renal function, or hypertension. So one should monitor a patient with class-II lupus nephritis for these changes. If the patient's clinical status does decline, a repeat renal biopsy is indicated to document whether class-III or -IV disease has developed. If this is the case, treatment with cytotoxic drugs should be started. Prescription of cytotoxic drugs to every patient with class-II lupus nephritis leads to overtreatment in the majority of these patients; the greatest proportion respond adequately to prednisone monotherapy.

DR. FERNANDO VALDERRÁBANO (*Professor of Medicine, Head, Department of Nephrology, Hospital General Universitario Gregorio Marañón, Madrid, Spain*): You showed clearly that lupus activity decreases in patients with SLE when they develop end-stage renal disease, a decrease probably owing to depression of the immune response in severe renal failure. On the other hand, treatment with recombinant human erythropoietin can improve the immune response in patients with end-stage renal disease [185, 186]. In your experience, does erythropoietin therapy reactivate disease activity in lupus patients undergoing dialysis?

DR. BERDEN: I have never seen this in our patients, nor am I aware of such an observation in the literature. Has anyone in the audience had such an experience?

DR. VALDERRÁBANO: We have monitored five hemodialysis patients in whom lupus nephritis was the primary renal disease. Erythropoietin therapy did not stimulate disease activity in any of them; followup was 6 to 36 months. Also, a 1994 report from Hebert et al found that administration of recombinant erythropoietin did not alter serologic parameters (C3 levels, ANA,

anti-dsDNA), or clinical disease markers (serum creatinine, urinalysis, proteinuria) in five SLE patients who had slightly impaired renal function (serum creatinine 150 μmol/liter, 1.7 mg/dl) [187].

DR. K.S. NAYAK (*Chief of Nephrology, Appollo Hospitals, Hyderabad, India*): You said that you performed a renal biopsy in your patient initially, and you found a low chronicity index and a very high activity index. Why did you not repeat the biopsy, especially before transplantation?

DR. BERDEN: We have one prerequisite for performing a repeat biopsy: the results of that biopsy should have therapeutic consequences. If you already are using the most extensive treatment protocol, a repeat biopsy is indicated only if you plan to stop the treatment if extensive chronic lesions (WHO class VI) are found. Our patient did not want to continue extensive immunosuppressive treatment after her fourth renal flare. She accepted the eventual development of end-stage renal disease, and hoped for successful renal transplantation, as a living kidney donor was available. In this instance, a repeat biopsy thus would have had no therapeutic consequences.

DR. DIMITRIOS GREKAS (*Associate Professor of Nephrology, University Hospital Ahepa, Thessaloniki, Greece*): Do you continue the every-three-month pulse therapy with intravenous cyclophosphamide for a finite number of years or indefinitely?

DR. BERDEN: In the Dutch protocol, it is continued for two years in total. We give the cyclophosphamide every month for six months, and then administer it every 3 months for 18 months. In the NIH protocol, these intravenous cyclophosphamide pulses are given for a maximum of 30 months [115]. Because the side effects of cyclophosphamide, especially its carcinogenic potential, urothelial cell toxicity, and risk of infertility, are related to the cumulative dose [108, 112], my advice would be not to exceed a treatment period of 30 months.

DR. GREKAS: What is your view concerning pregnancy in a young woman with SLE nephritis?

DR. BERDEN: I do not have the time in this Forum to present a balanced view on this topic. We would have to take into account all the different aspects, such as the effect of pregnancy on SLE, the effect of SLE on pregnancy, the impact of the anti-phospholipid syndrome on pregnancy, the neonatal lupus syndrome, and the consequences of pregnancy for immunuosuppressive treatment and vice-versa. This discussion by itself could be the subject of a Nephrology Forum. So let me offer our general policy, which is more or less identical to that for women with other non-lupus renal diseases. If the SLE is serologically and clinically inactive, the patient has good renal function, a normal blood pressure, and no nephrotic-range proteinuria, we do not think that pregnancy is contraindicated. But if her disease is active, the serum creatinine exceeds 150 μmol/liter (1.7 mg/dl), or hypertension is present, we strongly advise the patient not to become pregnant. Historically, physicians have feared that pregnancy or delivery will reactivate SLE. But a recent review concluded that the number of patients who experienced a disease flare during pregnancy is very low if the disease is inactive at conception [188].

DR. MAHER RAMZY (*Professor of Renal Medicine, Cairo University, Cairo, Egypt*): In your opinion, what is the most sensitive serologic marker for disease monitoring, ANA, anti-dsDNA, C3, or C4? My second question is: is there a difference in lupus between male and female patients?

DR. BERDEN: Reports from the Netherlands by Swaak et al [189–192] and by ter Borg and coworkers [6] showed that 80% to 90% of the disease flares are preceded by a significant rise in anti-dsDNA antibodies. These changes were more sensitive than were changes in complement profiles. These observations suggest that the anti-dsDNA titer is the best parameter for monitoring the patient. Of course, changes in C3 and C4 also can be taken into account, but these markers are less sensitive. As I said earlier, Kallenberg's group conducted a trial in which treatment was guided by changes in anti-dsDNA titers [125]. Another serologic marker that could be of potential interest for monitoring lupus nephritis is the anti-C1q antibody titer. The same group found that a rise in anti-C1q preceded a renal flare by 10 weeks [85]. Finally, future research will reveal whether measurement of nucleosome-specific autoantibodies will provide a better tool for disease monitoring. In view of the pathophysiologic concept that I presented, this approach seems promising.

Now let me respond to your second question. In a retrospective analysis, Miller et al discerned no differences in disease manifestations, disease severity, or positive family history for autoimmune diseases when they compared 51 male patients with female lupus patients [193]. In a more recent review, Kaufmann and colleagues noted a higher incidence of thrombocytopenia and renal disease in male lupus patients; all other clinical and serologic parameters did not differ [194]. As I already mentioned, the risk of developing ESRF is higher in male patients with lupus nephritis than in females.

DR. HERMINIO SUAZO (*Nephrologist, Kidney and Hypertension Center, Houma, Louisiana, USA*): According to the case summary, when the patient's serum creatinine reached 620 μmol/liter (7.0 mg/dl), she was started on a low-protein diet. Would you please comment on the evidence that dietary protein restriction is beneficial in nondiabetic patients with chronic renal disease as opposed to the risk of malnutrition in patients starting dialysis?

DR. BERDEN: The low-protein diet was not prescribed in an attempt to influence the progression of her renal disease, but because her urea levels were increasing and we wanted to control her azotemia. We prescribed 0.8 g of protein/kg body weight, an amount that generally does not lead to malnutrition.

DR. MARIANO RODRIGUEZ (*Associate Professor of Medicine, Hospital Universitario Reina Sofia, Cordoba, Spain*): Let me return to the question of pathogenesis. You mentioned apoptosis as a key causative factor in lupus. Has your group or any other group demonstrated that peripheral blood mononuclear cells show signs of apoptosis in vitro? If this is the case, could these cells be used to monitor treatment or detect flares?

DR. BERDEN: As I mentioned earlier, some observations in the literature point to a disturbed apoptosis in lupus. These include an increased spontaneous apoptosis in vitro [31, 195], an increased expression of Bcl-2 in lymphocytes [42] and, although not uniformly confirmed [40, 41], increased circulating levels of soluble Fas [39]. Kalden's group recently reported that phagocytosis of apoptotic cells was impaired in lupus patients [196]. However, none of these parameters has yet been evaluated for its diagnostic or prognostic potential. At present, Dr. Smeenk and colleagues (CLB, Amsterdam) are performing a prospective study to analyze the relation between levels of soluble Fas and disease activity. Their preliminary data indicate that disease flares are preceded, by as early as 5 months, by an increase of soluble Fas, while in patients with inactive disease, the levels remain normal. In the

near future, we will measure the surface expression of phosphatidylserine on peripheral blood lymphocytes, as this is one of the first signs of apoptosis at the cell surface. Other cell-surface changes that are not yet well-defined [197] also occur on apoptotic cells. As soon as these changes are elucidated, their significance for SLE can be probed. Finally, since nucleosome release is a key feature of apoptosis, measurement of nucleosome autoantibody complexes in the circulation of SLE patients could form a new tool for monitoring. This approach is now feasible because Fournié recently developed a method of measuring these complexes [198].

Dr. Zbigniew Hruby (*Associate Professor of Nephrology, Wroclaw School of Medicine, Wroclaw*): I would like to address the issue of flares or relapses that are refractory to treatment. Do you think that there is a role for intravenous immunoglobulin for these severe cases? Yesterday at this EDTA/ERA Congress Dr. Lockwood spoke about the therapeutic potential of intravenous immunoglobulin for the management of severe ANCA-associated vasculitides. Today you mentioned anti-GBM autoantibodies that could be inhibited by anti-idiotypic antibodies present in these immunoglobulin preparations.

Dr. Berden: Let me first make sure that my message was clear. No anti-GBM antibodies are present in SLE. In our concept, autoantibodies are targeted to the GBM, because they are complexed in the circulation to nucleosomes, thereby gaining affinity for the GBM. I do not have personal experience with intravenous immunoglobulin treatment in lupus nephritis. The literature contains only a few scattered reports on intravenous immunoglobulin therapy in lupus and no controlled data [199, 200]. In some patients intravenous immunoglobulin therapy had a brief but positive effect on extrarenal symptoms. Some patients even experienced an exacerbation of glomerulonephritis [199]. I do not think that I can recommend this form of treatment on the basis of these results.

Dr. Aïda R. J. Moussali (*Head of Nephrology Service, Hotel-Dieu de France Hospital, Beyrouth, Lebanon*): Do you envision a role for subcutaneously given heparin in the treatment of lupus, based on your experimental animal data?

Dr. Berden: In our experimental animal studies, if we started early with heparin and non-coagulant heparin derivatives, we could prevent proteinuria and could decrease the severity of the glomerulonephritis, as assessed by light microscopy and immunofluorescence. However, if we treated animals with already established proteinuria and nephritis, we did not observe a reduction in proteinuria, although the progression of proteinuria slowed. If we added non-coagulant heparin derivatives to a subtherapeutic dose of cyclophosphamide, we could prolong survival significantly compared to that with cyclosphamide treatment alone [70]. However, at present no data are available for human lupus. It nevertheless remains an intruiging possibility, moreover because heparin and heparinoids might have other beneficial effects on immune-mediated glomerular pathology, including inhibition of mesangial proliferation and sclerosis, modulation of extracellular matrix production, inhibition of binding of immune complexes to mesangial cells (at least in vitro), inhibition of complement activation, and downregulation of T-cell-mediated responses [70].

Dr. Moussali: I have another question regarding the patient who was presented. What were the odds of her becoming pregnant given the severity of her disease? In my opinion, her disease was so severe that the odds for her completing a successful pregnancy were poor. One more question: did she become pregnant after renal transplantation?

Dr. Berden: I agree with you that the disease in this patient was severe; it led to end-stage renal failure within four years. But a lupus patient with this form of nephritis has an 80% chance of not developing end-stage renal failure. We were hoping that she would respond well to the treatment and that she could become pregnant later if she met the criteria I already mentioned in response to one of Dr. Grekas' questions. I agree with you that during her disease her chances for a successful pregnancy were low, but she rejected cyclophosphamide treatment because of its risk of inducing infertility. Your second question was about pregnancy after renal transplantation. The patient, now in good condition, also raised this question. A search of the literature revealed only three lupus patients who received transplants and who later became pregnant [201, 202]. Two of the three patients carried their pregnancies to term successfully. I think that at the moment there are no absolute contraindications for pregnancy in our patient.

Dr. Jan J. Weening (*Professor of Pathology, Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*): In your discussion on the mechanisms of induction of SLE, you mentioned the possible role of abnormal apoptosis and the appearance of cryptic epitopes. Recent studies from Ernst Gleichmann and our own group have provided evidence that similar mechanisms might be involved in SLE induced by drugs and heavy metals. As you know, a number of drugs and heavy metals such as gold and mercuric chloride can cause SLE in humans and a lupus-like syndrome in laboratory animals. Gleichmann's group showed that gold can interfere with protein folding during antigen processing and result in presentation of cryptic peptides instead of the usual dominant peptides [203]. The cryptic peptides are not recognized as self and elicit an autoimmune response. In addition, Aten from our group showed that mercury can change antigenic peptides in such a way that T-cell recognition is altered. Furthermore, Aten showed that mercuric chloride can cause apoptosis in lymphocytes and possibly lead to immune dysregulation; transfection of the Bcl-2 gene resulted in a reduction in mercury-induced apoptosis [204].

Dr. Berden: Thank you for this comment, which extends the data that I discussed.

Dr. R. Banks (*Nephrologist, Gloucester Royal Hospital, Gloucester, U.K.*): How long would you continue treatment with steroids and azathioprine in a stable, quiescent patient? Is it a life-long sentence?

Dr. Berden: This is a very important question but one that is difficult to answer. To my knowledge, no controlled studies are available in which the medication was electively stopped. Boumpas et al suggested that quarterly pulses of cyclophosphamide should be continued at least one year after remission of renal disease is reached [205]. However, these authors did not state whether all medication was stopped. In a retrospective analysis of 84 SLE patients, Ciruelo and coworkers found that after cessation of CPM (given either orally or intravenously), the cumulative incidence of relapse of glomerulonephritis was 25% at 5 years and 46% at 10 years [206]. Risk factors for relapse included oral treatment with CPM, onset of nephritis before the age of 29 years, and delay of treatment from onset of nephritis for more than five months. Similar data are not available for cessation

of treatment with prednisone and/or azathioprine. In a retrospective analysis in a relatively small number of patients (n = 25), Moroni et al found that after a mean followup of 88 months, 11 patients were stable without treatment. The incidence of lupus flare-ups decreased significantly after the tenth year (0.11 flares/patient/year versus 0.31 in the years before) [207]. Until recently, we treated our patients with low (alternate-day) doses of prednisone and azathioprine (1.5 mg/kg) for at least 10 years after onset of nephritis. In the ongoing Dutch prospective study, patients in both treatment arms receive after two years 10 mg of prednisone and 2 mg/kg azathioprine, both of which are continued for two more years. Thereafter, we taper the medication gradually using careful monitoring of clinical and serologic (anti-dsDNA, C3, and C4) parameters. The Dutch study will evaluate the consequences of this withdrawal policy.

DR. ALEXANDER CROWE (*Senior Registrar in Nephrology, Royal Liverpool University Hospital, Merseyside, U.K.*): The patient in the presentation received a renal transplant six months after starting renal replacement therapy and while her serologic markers were negative. What safety criteria do you use for the timing of transplantation with respect to lupus?

DR. BERDEN: The general policy is to transplant a kidney 6 to 12 months after the onset of ESRF, in the absence of lupus disease activity [127, 208]. However, this policy is based on a prudent approach rather than on hard data. No differences were found for graft or patient survival between patients who received transplants within or after three months of dialysis treatment [209]. Also Goss et al reported no clear benefit from a longer dialysis period [137]. In the Dutch study on transplantation in lupus patients, disease activity prior to transplantation did not influence patient or graft survival [135]. It has been suggested, but not unequivocally proven, that there is an association between pretransplant positive lupus serology and recurrence [137]. Therefore our approach is to wait for at least three months to allow for eventual recovery of renal function, and to perform transplantation after lupus serology has become negative.

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